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THE INVASIVE CHARACTER OF CANCER GROWTH. By DALE REX COMAN, M.D. . . 257

MULTIPLE PULMONARY HEMANGIOMATA. By PAUL TODD MAKLER, M.D., and DAVID ZION, M.D. 261

EFFECT OF STREPTOMYCIN ON EXPERIMENTAL BRUCELLOSIS IN GUINEA PIGS. By I. LIVE, F. G. SPERLING, and E. L. STUBBS. 267

THE TREATMENT OF ACUTE RHEUMATIC FEVER WITH LARGE DOSES OF SODIUM SALICYLATE. WITH SPECIAL REFERENCE TO DOSE MANAGEMENT AND TOXIC MANIFESTATIONS. By B. V. JAGER, M.D., and R. ALWAY, M.D. 273

EFFECT OF SODIUM BICARBONATE ON THE THERAPEUTIC EFFECTIVENESS OF SULFADIAZINE IN MICE. By ARTHUR KORNBERG. 286

PENICILLIN IN ACUTE NEPHRITIS IN CHILDREN. By SUSANTA SEN, M.A., B.Ch., M.D. (Cantab.), F.R.C.S. (Edin.), M.R.C.P. (Lond.) 289

PENICILLIN THERAPY IN ULCERATIVE COLITIS. A PRELIMINARY REPORT. By CAPT. BERNARD B. KOROSTOFF, M.C., A.U.S., and CAPT. HARRY E. KING, M.C., A.U.S. . 293

COMBINED PENICILLIN AND SULFADIAZINE THERAPY IN PNEUMOCOCCIC PNEUMONIA. By MORRIS F. COLLEN, M.D., ALVIN L. SELLERS, M.D., and ERIC C. KAST, M.D. . 299

THE INFLUENCE OF SULFANILAMIDE THERAPY UPON THE COURSE OF ACUTE GLOMERULONEPHRITIS IN CHILDREN. By MILTON RAPOPORT, M.D., MITCHELL I. RUBIN, M.D., and ARTHUR D. WALTZ, M.D. 307

THE TREATMENT OF TEMPORARY RENAL INSUFFICIENCY (UREMIA) BY PERITONEAL LAVAGE. By WILLIAM E. ABBOTT, M.D., and LIEUT. PATRICK SHEA, (MC) USNR . 312

RELATIVE EFFICIENCY OF QUINACRINE (ATABRINE) AND QUININE IN TREATMENT OF ACUTE ATTACKS OF VIVAX MALARIA. By MAJOR HARRY MOST, M.C., A.U.S., and COL. JOSEPH M. HAYMAN, Jr., M.C., A.U.S. 320

QUALITATIVE CHANGES IN FIBRINOGEN WHICH INFLUENCE THE ERYTHROCYTE SEDIMENTATION RATE AND THE CLOT RETRACTION TIME. By IRA R. MORRISON, M.D. 325

PERSISTENT VENTRICULAR BIGEMINAL RHYTHM IN APPARENTLY NORMAL HEARTS. By WILLIS SENSENBACH, M.D., and RODERICK M. BUIE, Jr., M.D. 332

FUNCTIONAL PAROXYSMAL AURICULAR FIBRILLATION. By CAPT. ARTHUR M. MASTER, (MC) USNR, and LIEUT. HERBERT EICHERT, (MC) USNR 336

THE EFFECT OF SODIUM SALICYLATE ON THE SEDIMENTATION RATE OF ERYTHROCYTES IN VIVO. By F. HOMBURGER, M.D. 346

PROGRESS OF MEDICAL SCIENCE:

PREVENTIVE MEDICINE AND EPIDEMIOLOGY: SEROLOGIC TYPES OF BACTERIA AS AN EPIDEMIOLOGIC PRINCIPLE. By W. LLOYD AYCOCK, M.D., and GEORGE E. FOLEY. 350

PATHOLOGY AND BACTERIOLOGY: THE SPREADING FACTORS AND BACTERIAL INFECTION. By DONALD S. FLEMING, M.D., D.P.H. 374

PROCEEDINGS OF THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA . . . 380

Contents continued on adv. page 10

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

MARCH, 1946

ORIGINAL ARTICLES

THE INVASIVE CHARACTER OF CANCER GROWTH*

By Dale Rex Coman, M.D.

PHILADELPHIA, PENNSYLVANIA

(From the Department of Pathology, University of Pennsylvania Medical School)

As my subject deals with the invasive character of cancer growth, it is well first to consider precisely what is meant by the term invasiveness.

Let me begin by reminding you that all tumors consist of cells derived from the body tissues. A tumor might be defined for our purposes as an excessive and limitless local multiplication of cells. This local multiplication leads to the formation of a lump, a swelling, a tumor.

Tumors may be divided into two main groups, benign and malignant. Benign tumors remain strictly localized; they are circumscribed and sharply demarcated from the surrounding normal tissues. Frequently a capsule forms around them, serving as a sort of mantle that separates the tumor cells within from the normal cells outside. As the cells of the benign tumor multiply the tumor grows larger, but its growth is expansive, like a balloon being inflated with air. Thus the benign tumor merely compresses and pushes aside the surrounding normal tissues.

Malignant tumors, however, do not remain strictly localized. The cells of the malignant tumor infiltrate and permeate into the surrounding parts. There is no capsule, or only an incomplete one. As the cells multiply the tumor increases in size, but not like a balloon expanding; rather, like the roots of a plant penetrating the soil. The cells of the malignant tumor disrupt the surrounding tissue, dislocating the normal cells and often destroying them. The cancer cells may even gain entrance to a blood vessel or a lymphatic vessel in this way and so be swept into the general circulation and then be deposited in some distant part of the body. Becoming established there by their continued multiplication they produce a secondary tumor, or metastasis.

It is this invasive type of growth which concerns us now. Upon what does this invasiveness depend? Why do benign tumor cells remain localized? What is it that enables malignant cells to penetrate the surrounding parts?

First, it is not the presence of a capsule that makes the benign tumor remain localized. Sometimes a benign tumor becomes malignant. When it does, it expresses its malignancy by invading through the capsule. The capsule, then, is no barrier to the invading cancer cells.

It was suggested long ago that malignant tumor cells might secrete some substance that would destroy the adjacent normal tissues, without injuring the tumor cells. Such heterolytic ferments were, indeed, reported by several investigators.[1,8,13,14] Further work,[10,12] however, did

* An address before the Philadelphia Chapter of the Sigma Xi, November 28, 1945.

not substantiate these reports and today this hypothesis is no longer seriously considered by most investigators.

A third attempt to explain invasive growth suggested that the tumor cells were forced into the surrounding tissues because of the pressure built up within the tumor by the proliferating mass of cells.[6] The tumor thus supposedly broke through its bonds and penetrated neighboring parts. The cells, once free, might further invade by their own ameboid movement. But this mechanism does not explain why benign tumors do not invade. Benign tumors may grow as large as malignant ones, and frequently become even larger because they do not invade and kill the host. Where one incises a benign tumor, often it bulges outward, indicating that the cells within the tumor were under considerable pressure, yet they were not forced out into the neighboring tissues.

This attempt at explanation, then, does not fit the facts. It remains true, however, that mechanical stresses and tensions do explain the mechanism of displacement of cells, *once they are free to be displaced.* Benign tumor cells are evidently bound in some way so that they are incapable of displacement, in contrast to malignant cells which are free. Because it is free, the malignant cell can be displaced mechanically or wander off by ameboid movement.

I shall mention a fourth explanation only because it has been frequently repeated. The statement is made that "Malignant cells invade because they have escaped from the formative influences of the neighboring tissues." This is obviously no explanation at all, because the term "formative influences" lacks definite meaning. It would obstruct further investigation if we folded our hands in acceptance of such a statement.

It has been suggested that the malignant tumor is invasive because its growth is peripheral;[7,9] that is, the cells of the tumor which multiply are at the edges of the tumor instead of at the center as in benign tumor. But mitotic activity occurs throughout both benign and malignant tumors. It becomes less conspicuous in the central part of any tumor, benign or malignant, in which part the blood supply is reduced by the increasing size of the tumor. This suggestion, then, is not a satisfactory explanation of invasive growth.

To summarize at this point:

The benign tumor does not remain localized because of the presence of a capsule.

The suggestion that malignant cells secrete destructive ferments has not been supported by evidence.

The explanation that malignant cells are forced into the neighboring tissues by pressure within the tumor, due to cell multiplication, leaves unanswered the question, why then do benign tumors not invade?

The explanation that malignant cells have escaped "local formative influences" is no explanation at all.

Last to be mentioned, the suggestion that invasiveness depends upon peripheral cell multiplication in malignant tumors, whereas proliferation in benign tumors is central, fails because multiplication occurs in both types throughout.

What other possibilities are there?

A year or so ago the following observation was made.[4] An attached pair of fresh living cancer cells from a man's lip were examined with a micromanipulator. One micro needle was inserted into one of the cancer cells, the other into the other cell. The needles were then moved gradually apart. It was noted that the cancer cells separated, one from the other, very easily. But when a pair of normal cells from the lip were treated in the same way, they clung to each other tenaciously.

This observation suggested a possible solution of our problem. If it were found that malignant cells were indeed easily separable one from the other, it would offer a physical basis for invasive growth. Such easily detached cells would then be free for mechanical displacement into the surrounding tissues, or free to wander off by their own locomotion.

Therefore, a large number of cells was

examined with the micromanipulator. Cells were obtained from cancers of the lip, from normal lips, from cancers of the cervix, from normal cervices and from papillomas of the skin (benign tumors). The technique was refined so that the force required to pull apart a pair of cells could be measured. This was accomplished by measuring the bend produced in a microneedle as it pulled a pair of cells apart. One stiff unbending needle held one of an attached pair of cells. The tip of the other needle was inserted into the other cell. This latter needle was flexible and had been calibrated previously with microweights, so that it was known that a given force would produce a certain amount of bending. By measuring the amount of bend in the needle, the force necessary to pull a pair of cells apart was obtained. The results of this investigation are shown in Table 1. Malignant cells, whether from the lip or cervix consistently showed low values of cohesiveness as compared with normal and benign tumor cells.

TABLE 1.—FORCES REQUIRED TO SEPARATE PAIRS OF CELLS BY MICROMANIPULATION

Derivation of cells	Mean and its standard error, mg.
Normal lip	1.42 ± 0.041
Carcinoma, lip	0.47 ± 0.051
Papilloma, skin	1.25 ± 0.032
Normal cervix	1.11 ± 0.039
Carcinoma, cervix	0.18 ± 0.022

Each figure is based upon 50 pairs of cells and represents the mean (with its standard error) of the force in mg. required to separate the cells by micromanipulation. It is seen that the values for carcinomatous cells are much lower than those for normal cells and for cells from papillomas.

Thus is would seem from these observations that cancer cells are indeed less firmly attached to one another than are normal or benign tumor cells, and as mentioned before, this offers a physical basis for invasive growth.

But, we must go further. Upon what does this cohesiveness of cells depend? It seems most likely that it must in some way depend upon the state of the cement substance that is known to exist between cells and which serves to bind them together. Can we find any differences between benign and malignant tumor cells in regard to this cement substance?

Two possible explanations will be presented. The first of these we shall call the calcium deficiency hypothesis.

Biologists have known for a long time that calcium plays a part in maintaining the cohesiveness of cells. Cells which normally stick tightly together will separate readily if calcium is withdrawn from the medium in which the cells are immersed. The stability of the intercellular cement substance is thus dependent upon the presence of calcium.[15]

Supposing then that it could be shown that cancers were deficient in calcium, this might lessen the cohesiveness of the cells and lead to invasive growth as suggested above.

Carruthers and Suntzeff[3] applied a carcinogenic chemical to the skin of mice. They then measured the calcium content of the normal skin of mice and compared it to the calcium content of the skin after treatment with the chemical carcinogen. They found that soon after the application of the carcinogen, the calcium content dropped. Another large drop in calcium occurred when the skin cells became truly cancerous and invasive. Furthermore a group of human cancers was analyzed by Scott[16] who found that they were deficient in calcium. This calcium deficiency in cancer bearing patients is a local deficiency, confined to the tumor; the blood level of calcium in cancer patients is not disturbed.

The calcium deficiency hypothesis, then, finds support in the chemical analysis of cancer tissue. It may be the answer to our question, though it leaves for the future the next question, why do cancer cells contain less calcium?

The second possible explanation of the decreased cohesiveness of cancer cells we shall call the increased spreading factor hypothesis.

It is a property of some substances[5] to enhance the spread or diffusion of small particles or solutions through living tissues. Such substances are called spreading

factors. A very potent spreading factor is an enzyme called hyaluronidase, which can be extracted from testicular tissue. If a solution of hyaluronidase is mixed with India ink and then injected into the skin of a rabbit it causes the ink particles to spread widely through the skin. The action of hyaluronidase is to hydrolyze hyaluronic acid,[11] one of the chief constituents of the intercellular cement substance, thereby separating the cells and allowing the ink particles to pass between them.

Is it possible that some such mechanism as this plays a part in causing the decreased cohesiveness of cancer cells? If so, cancers should have a high content of spreading factor.

Boyland and McClean[2] determined the spreading factor content of a large group of animal tumors. These tumors ranged from benign, non-invasive tumors to malignant, invasive cancers. They reported that the spreading factor content paralleled the malignancy of these tumors; the more malignant the tumor the more spreading factor it contained.

This result would certainly seem to lend support to the hypothesis that the decreased cohesiveness of cancer cells depends upon an excessive amount of spreading factor. Furthermore, if this substance were released by the cancer cells it might act upon the cement substance of the adjacent normal tissues, thus facilitating the invasion of the normal tissues by the cancer cells. The work of Boyland and McClean has not as yet been confirmed elsewhere. A similar analysis of human tumors is under way in our laboratory at the present time, but has not progressed sufficiently far to allow conclusions to be drawn.

This discussion began with a single question, upon what does the invasive growth of cancer depend? Instead of a single answer, I must close with several questions.

Does the decreased cohesiveness of cancer cells depend upon the local calcium deficiency that is known to exist in cancer tissue? Or, does it depend upon a local increase in spreading factor? May it not in some way depend in part upon each of these factors? Perhaps the actions of calcium, hyaluronic acid, and hyaluronidase are in some way connected in regulating the consistency of the cement substance. And finally, are there any other ways in which the invasive growth of cancer cells can be explained?

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MULTIPLE PULMONARY HEMANGIOMATA

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Within recent years, the attention of medical men has been increasingly directed to diseases of the chest by the advent of successful thoracic surgery. Because of the increasing interest in the accurate diagnosis of intrathoracic lesions, we are reporting, in this paper, a new case of pulmonary hemangioma. The previously reported cases of this condition, 7 in number,[2–4,6–9] will be briefly reviewed in order to call attention to the fact that pulmonary hemangioma produces so characteristic a syndrome that it may be strongly suspected from clinical evidence alone.

In 1938, Rodes reported the first case of pulmonary hemangioma with polycythemia and other evidences of chronic anoxemia.[8] The patient had died of massive hemorrhage apparently spontaneous in nature, and the lesion was demonstrated postmortem. The fact that this condition is truly rare, and that the paucity of reports is not merely the result of faulty diagnosis, is indicated by the fact that large series of routine autopsies throughout the world make no mention of this condition.

A pulmonary hemangioma (also called pulmonary arteriovenous fistula) is a knot of blood vessels connected by feeder vessels with both the pulmonary arterial and venous circulations. Thus it acts as a shunt whereby blood can pass from the arterial to the venous circulations of the pulmonary circuit without passing through the lungs and being oxygenated. The physiologic effect of this is to produce a low oxygen saturation of the arterial blood supply; all other changes are secondary to this anoxemia. The tumors themselves are cavernous hemangiomata, benign in nature.

Case Report. In November of 1944, the descriptions of pulmonary hemangiomata that had appeared in the literature since 1938[3,4,6,7,8,9] called to mind a patient seen in 1941. This young man was asked to present himself for further examination. He is native born of Italian descent, who was a patient of Dr. Jacob Makler in 1941. At that time he was 17 years old. His complaints of 3 years duration, were marked cyanosis and mild dyspnea on exertion. His family history was negative. He was not a "blue baby" at birth. There had been no severe pulmonary disease in infancy and there had never been a history of rheumatic fever. He had never lived at high altitudes. There had never been known exposures to silver salts, arsenic, aniline, manganese, or coal tar derivatives. His growth and development had been normal. Physical examination revealed an asthenic but normally built man. He had marked cyanosis. The fingers were markedly clubbed. The lungs and heart were completely normal to examination. Neither the liver nor the spleen were palpable. Temperature, pulse and respiration were normal and the blood pressure was within normal limits. The laboratory studies and special examinations done at this time are presented in Table 1. There was slight polycythemia. The white count, venous pressure, circulation times and electrocardiogram were all normal. A Roentgen ray of the chest was interpreted as showing increased vascular shadows probably due to the polycythemia. Otherwise the film was normal. The patient was discharged with the diagnosis of congenital heart disease, probably intra-auricular septal defect; but, it was understood at the time, the diagnosis was not satisfactory because a right to left shunt sufficient to cause marked cyanosis should cause murmurs, evidence of right ventricular enlargement or other signs of cardiac disease.

In 1941 a Roentgen ray of the chest was taken as a follow-up procedure. The film

was reported by Dr. Arthur Finklestein as showing increased hilar markings probably due to the polycythemia. A note was made to the effect that the shadows might be caused by the presence of abnormal blood vessels, but no further attention was paid to this possibility.

When the patient returned to us in November 1944, he reported that he had had no new symptoms since his previous admission with the exception of occasional nosebleeds and headaches. The cyanosis, clubbing and dyspnea were very prominent but he did not think that they were becoming worse. He was leading a normal life and was attending college at the time. The physical examination was very much the same as on the first admission with two exceptions. A small telangiectasis was present behind the right ear and a systolic murmur was heard in the left fourth interspace, 5 cm. to the left to the mid-sternal line. The great possibility is that the telangiectasis was missed on his first physical examination, but we have no way of telling whether or not the murmur is a recent development.

Roentgen Findings. In our original examination in 1941, a fairly discrete opacity measuring approximately 2 cm. in diameter was noted in the mid-portion of the right lung field at the level of the eighth rib posteriorly. A second poorly defined, irregular opacity measuring 3 cm. in diameter was noted in the seventh posterior interspace in the mid-portion of the left lung. From each of these opacities, a broad linear shadow was seen extending towards the hilum. The roentgen interpretation, at this time, was that the changes noted were due to thrombotic vessels secondary to the polycythemia. We were not satisfied with this interpretation and reëxamination at a future date was suggested for comparison.

Eight months following the original study, the patient returned and was re-rayed. There was no significant change in the previously described lesions. The heart was again of normal size and configuration. At this time possibility of angioma was suggested although the original diagnosis was not changed.

The present studies, done in November 1944, showed two additional masses similar to those previously described. They lay in the right lung and one was well down into the shadow of the diaphragm. The heart was still normal in size and configuration. The originally described opacities were again seen. The linear shadows extending to the hilum were interpreted as feeder vessels to the angiomatous masses. During the chamber analysis, under fluoroscopic observation, the intrathoracic pressure was altered by asking the patient to try to expire with the mouth closed and the nostrils occluded (Valsalva experiment), and then to try to inspire under the same conditions. These efforts resulted in a marked decrease and then increase in the size of the masses and feeder vessels, as the negative pressure was made lower and then increased. Repeated attempts at long exposure films were not successful in demonstrating pulsation of the masses. Laminographic studies were then done to clearly delineate the areas involved. The shadows extending from the hilar region were seen to be dilated pulmonary vessels. The lung field masses presented an irregular worm-like configuration that was continuous with the feeder vessels (see illustration). These findings confirmed the previous impression of multiple pulmonary hemangiomata.

It will be seen (Table 1) that there had been progression of the abnormal findings present on the first admission. The red count and hemoglobin were higher. The circulation times were slower, as would be expected in view of the increased red cell count. The sternal marrow aspiration showed a slight tendency to overactivity of erythropoiesis. The ratio of red cells to white cells was about 1:1, and this is the upper limit of normal for the red series. The marrow, in general, was normally cellular.

In summary, then, we had a young man in good health except for polycythemia and the signs and symptoms referable to it. These were cyanosis, clubbing of fingers, headache, easy fatigue, nosebleed, and slight dyspnea on exertion.

In addition, he had a peculiar murmur in the fourth interspace 5 cm. to the left of the mid-line, and had a small telangiectasis behind one ear.

At the time of this writing, November 1945, the patient has remained clinically unchanged since his last hospital admission a year ago.

Differential Diagnosis. The causes of polycythemia may be given as:

1. Polycythemia vera
2. Secondary polycythemia
   - *a.* High altitude
   - *b.* Poisoning by heavy metals or aniline dyes
   - *c.* Pulmonary disease preventing adequate oxygenation
     - Ayerza's disease
     - Emphysema
   - *d.* Cardiac anomalies with a right to left shunt
     - Tetralogy of Fallot
     - Complex of Eisenmenger
     - Pulmonary artery stenosis with septal defect
   - *e.* Pulmonary hemangioma

all indicate that the polycythemia is secondary rather than primary.

Considering the list of causes of secondary polycythemia, we were able to eliminate several items by history alone. The patient had never dwelt at high altitudes; and, in any case, it has been demonstrated[5] that the polycythemia caused by this practice persists for only a short time after the subject has returned to sea level. Poisoning by heavy metals or aniline dyes was very unlikely since the patient was aware of no prolonged exposure to these substances. The prolonged course of illness is also against such poisoning being causative. The possibility of the existence of chronic pulmonary disease was next to be considered. Such disease might well account for the anoxemia that stimulates the marrow to produce abnormally large numbers of red cells. Our patient had a normally shaped thorax with ample and symmetrical expansion during

TABLE 1.—LABORATORY STUDIES

	September 11, 1941	November 18, 1944	
Red cell count	6,200,000	7,700,000	
Hemoglobin	17 gm.	19.5 gm.	
White blood count	7900	6500	
Polymorphonuclears	67%	69%	
Eosinophils	1%	0%	
Basophils	0%	0%	
Lymphocytes	28%	28%	
Monocytes	4%	3%	
Hematocrit	Not done	55	
Platelet	Not done	175,000 (normal for this lab.)	
MCV	....	71cμ	
MCH	27 γ	25 γ	
MCHC	....	35%	
Venous pressure	125 mm.	125 mm.	
Calcium time	14 sec.	20 sec.	
Ether time	9 sec.	14 sec.	
Sternal marrow	Not done		
Erythrogones		2	
Normoblasts A		5	
Normoblasts B		11	
Normoblasts C		26	
		—	Total R.B.C. 44
Blasts		1	
Promyelocytes		1	
Myelocytes		7	
Metamyelocytes		17	
Mature polymorphonuclears		19	
Eosinophils		3	
Monocytes		2	
Megakaryocytes		0	Granulocytes 50
Lymphocytes		6	Lymphocytes 6
			100

In the case of our patient, there was almost nothing to suggest a diagnosis of polycythemia vera. His youth, the lack of splenomegaly, the normal range of the white cell and platelet counts, and the lack of hyperplasia of the white cell progenitors and megakaryocytes in the bone marrow

inspiration. The diaphragm appeared on physical examination to descend amply. The breath sounds were normal throughout the chest and there was no alteration of the percussion note. Roentgen examination, including fluoroscopy confirmed these findings. Furthermore, the absence of right ventricular enlargement on physical examination, on electrocardiographic examination and on Roentgen ray study was strongly against any great amount of emphysema or Ayerza's disease.

FIG. 1.—Laminographic Roentgen ray: a pulmonary hemangioma is seen in the left lung field with its feeder vessel going to the hilum. A descending feeder vessel is poorly seen in the right lung field, paralleling the mediastinal shadow.

Perhaps the most difficult and important cause of polycythemia to rule out was congenital heart disease. Here again we were forced to utilize negative findings for the most part. The congenital heart lesions that cause anoxemia, cyanosis, clubbing and polycythemia are those associated with a very large shunt of blood from the right side of the heart or great vessels to the left side without the blood passing through the lungs. It has been estimated[10] that 30% of the blood must pass through such a shunt in order for cyanosis to occur. Conditions that might cause such a deviation in the normal blood flow are absence of the interventricular septum, gross septal defects with pulmonary stenosis, and transposition of the great vessels. In all these conditions, one obtains a history of cyanosis from birth, the patient's growth and development are often seriously impaired, and cardiac enlarge-

ment with preponderance of the right ventricle is demonstrated by physical examination, Roentgen ray of the chest and right axis deviation on the electrocardiogram. Furthermore characteristic murmurs can usually be demonstrated. The absence of the above named factors made it seem very unlikely that congenital heart disease was the cause of our patient's secondary polycythemia.

The elimination of the usual causes for polycythemia left us with strong suspicion that pulmonary hemangioma was the correct diagnosis. Accordingly, Roentgen ray confirmation was sought by means of fluoroscopy and laminography as well as the customary technique for chest films. The complete report appears in the case report.

Table 2.—Analysis of 7 Reported Cases

Cases reported	(1)	(2)	(3)	(4)	(5)	(6)	Present case
Age	25	40	23	22	30	24	20
Dyspnea	+	+	+	+	+	+	+
Clubbing	+	+	+	+	+	+	+
Cyanosis	+	+	+	+	+	+	+
Hemangiometa elsewhere	+	0	0	+	+	0	+
Cardiac disease	0	0	0	0	0	0	0
Lung disease	0	0	0	0	0	0	0
R.B.C. in millions	7.5	6.5	9.5	11.5	..	7.5	7.7
Number of lung hemangiomata	3	1	1	1	3	1	4
Hemorrhage	+	0	0	0	+	0	0
Course	Death from hemorrhage	Well	Pneumonectomy, well	Well	Multiple excision after hemorrhage, well	Pneumonectomy, well	Well

Discussion. In view of the results in the other cases of this disease, the prognosis must be accepted as grave, with the danger of massive hemorrhage always present. Even if this does not occur, polycythemia is a condition that is associated with serious complications. Exhaustion of the marrow with anemia, granulocytopenia, thrombocytopenia or any combination of these conditions may occur. It has been stated that leukemia or erythroleukemia is a complication of polycythemia, but this is not generally accepted. A peripheral blood picture resembling chronic myelogenous leukemia can occur when the marrow becomes exhausted and myeloid metaplasia of the spleen occurs. Under such circumstances, nucleated red cells, and immature white cells, including myelocytes, may appear in the blood. These factors, plus the anemia and thrombocytopenia and the enlarged spleen, cause a superficial resemblance to chronic myelogenous leukemia. However, the marrow

Review of Previously Reported Cases. So few cases of this nature have been reported that this summary can draw no sweeping conclusions. A reference to Table 2 will show that the cases have been remarkably similar. We believe that this similarity is so striking that a definite clinical picture may be said to exist. In view of the far greater frequency of congenital cardiac lesions as a cause for polycythemia and cyanosis in the young, pulmonary hemangioma can only be kept in mind until the heart has been ruled out as the source of trouble. All the cases reported have been young adults. Dyspnea, clubbing, cyanosis and polycythemia have been common to all, although the case of Janes[6] does not mention the red count. None of the 7 cases have had demonstrable heart or lung disease. Three of the 7 cases had multiple pulmonary hemangiomata, our case having the largest number. Four of the 7 cases had demonstrable hemangiomata elsewhere. Hemorrhage occurred in 2 of the cases and has also been reported as the cause of death in an infant who had a pulmonary hemangioma and died at such an early age that the picture of the chronic condition could not occur.[2]

shows exhaustion and aplasia instead of the hyperplastic state seen in leukemia.

In any polycythemic patient, thrombosis is a great danger and may even cause death. The increased viscosity of the blood, which results from increase in the red cell mass without adequate concomitant increase in the plasma,[5] results in slowing of the circulation and an increased tendency to clot.

It must also be considered that the increased viscosity of the blood causes an abnormal burden to be placed upon the heart.

Lastly, duodenal ulcer is found commonly in patients together with polycythemia,[1] and the latter may possibly predispose a patient to the former by producing vascular changes and plugging of small vessels in the intestinal wall.

In view of all these factors, it is probable that surgery is the best method of handling cases of this disease. The extent and type of the surgery naturally depend upon the particular case. Local excision, lobectomy and pneumonectomy are all possible. In the patient with whom we are now concerned, the multiplicity of the lesions, and their bilateral distribution, make operation hazardous. The patient has been informed of the situation and does not wish surgery at this time. The possibility of operation is still being considered for some future time. No attempts to depress the formation of red cells by Roentgen ray, radioactive phosphorus or other methods are contemplated, since their efficacy is questioned and their risk is not. Phlebotomy for the relief of symptoms will be undertaken if there continues to be progression of the polycythemia, headache, and so forth.

Summary. A case of pulmonary hemangioma is presented with a clinical picture sufficiently typical for a clinical diagnosis to be made. An examination of the sternal marrow, and a fluoroscopic study of the pulmonary lesions are reported. We believe this to be the first time these procedures have been done in a case of this nature. The changes in the lesions during a Valsalva experiment are of particular interest, being reported for the first time.

The literature on this subject is briefly summarized. The importance of thorough Roentgen ray study in assessing the nature and extent of the lesion is emphasized. It is stressed that this lesion should be carefully sought for in obscure cases of polycythemia, cyanosis, hemoptysis, murmurs, or abnormal shadows on Roentgen ray, since surgical cure can be effected in a fair number of instances. A brief discussion of the differential diagnosis and complications of polycythemia is included.

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EFFECT OF STREPTOMYCIN ON EXPERIMENTAL BRUCELLOSIS IN GUINEA PIGS*

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There is no known effective treatment of brucellosis. In discussing the use of sulfonamides in veterinary practice, Stableforth[7] states that they have not been found of value in the treatment of *Brucella abortus* infections, and according to our own experience, the administration of sulfapyridine[4] and sulfathiazole[5] to cows suffering with brucellosis was ineffective. Keefer[3] in reviewing recently the status of penicillin in the treatment of infections, lists Brucella among the microörganisms which are not susceptible to the action of this antibiotic substance. On the other hand, streptomycin,[6] a substance isolated from *Actinomycetes griseus*, has been found by Jones, Metzger, Schatz and Waksman[2] to protect chick embryos against infection with *B. abortus*. On the basis of these findings it was decided to investigate the effect of streptomycin on experimental infection of guinea pigs with *B. abortus*.

Experiment 1. Eighty guinea pigs were infected with *B. abortus;* 40 were then treated with streptomycin, and the others served as controls. Each of the guinea pigs was injected subcutaneously with 20,000 *B. abortus* as determined by plate counts which represented at least 1000 infective doses because 2 guinea pigs injected at the same time with a 1000-fold dilution of this concentration, *i. e.*, 20 Brucella cells, were positive on cultures when autopsied 4 weeks later. The culture used was an anaërobic strain of *B. abortus* which was originally isolated from the milk of a naturally infected cow, and was subsequently passed through guinea pigs four times, thus enhancing its virulence. Finally, the culture was lyophilized to preserve its virulence, and was subcultured 48 hours prior to infection of the animals. The weights of the guinea pigs before infection ranged between 350 and 500 gm., and they were weighed weekly following infection.

Dosage. In treating tuberculosis in guinea pigs, Feldman and Hinshaw[1] found that the more purified the streptomycin the better it was tolerated by the animals, and they used daily doses ranging from 1750 to 6000 units.† Throughout this experiment the treated guinea pigs received 5000 units daily divided into 5 equal doses. Each dose of the material was dissolved in 1 ml. of sterile distilled water, and the injections were made subcutaneously at 3-hour intervals beginning at 9 A.M. and ending at 9 P.M. each day. Administration of streptomycin to 20 guinea pigs was begun 3 hours after infection, and to 20 other guinea pigs 1 week after infection.

Studies in Vitro. By keeping the number of *B. abortus* organisms constant (approx. 10 million) and varying the concentration of streptomycin, it was found that 1.2 units were required per ml. of tryptose agar or tryptose broth to inhibit completely the growth of the organisms. In addition, this concentration of streptomycin in tryptose broth proved definitely

* These studies were supported by funds from the Pennsylvania Department of Agriculture and the Bureau of Animal Industry, United States Department of Agriculture. The streptomycin was provided through the courtesy of Merck & Co.

† A unit of streptomycin is that quantity of the antibiotic agent which inhibits the growth of a given strain of *Escherichia coli* in 1 ml. of nutrient broth or agar.

bactericidal because no growth was obtained when 0.5 ml. quantities of the broth-streptomycin-Brucella mixture were subcultured on pour plates after 18 hours incubation, while subcultures of the controls yielded an abundance of growth. Similar results were obtained with the use of a 100-fold dilution of the organisms (approx. 100,000), which would indicate that when a sufficient quantity of streptomycin was present in the culture medium, it killed a high as well as a low concentration of Brucella organisms. Five strains of *B. abortus*, 3 aërobic and 2 anaërobic ones, were thus tested, and no appreciable difference was noticed in the mode of streptomycin action upon them.

Toxicity. The animals tolerated the streptomycin well, and no general or local toxic effects were noticed in connection with the administration of the material. There were some slight differences in the weight increase of the animals in the various groups at the end of 4 weeks; *i. e.*, the average gain in weight per guinea pig in the group treated for 27 days was 89.5 gm.; in the group treated for 20 days, 108.6 gm.; and in the non-treated infected group, 85.9 gm. Hemoglobin determinations on the blood prior to autopsy of the animals showed an average of 13.5 gm. of hemoglobin per 100 ml. of blood in the treated groups, and 13.6 gm. per 100 ml. of blood in the untreated infected controls.

Results of Therapy. The administration of streptomycin was discontinued 27 days after infection. Thus, one group of 19 guinea pigs (one animal died of intercurrent disease), had been treated for 27 days and another group of 20 guinea pigs for 20 days. Blood was obtained for agglutinative tests and hemoglobin determinations 24 hours after cessation of treatment from 9 guinea pigs which had been treated for 27 days, from 10 guinea pigs which had been treated for 20 days and from 19 controls, and then these animals were killed and their tissues cultured. Eight days after therapy was discontinued, blood was obtained from the remaining animals, and then they were killed and their tissues cultured. In each instance one of the inguinal lymph glands and pieces of liver and spleen were cultured by rubbing the cut surface of each tissue over a separate tryptose agar plate, and the plates were incubated under 10 per cent CO_2 tension for 5 days. One treated guinea pig and 3 controls died of intercurrent disease during the early part of the experiment, and are, therefore, not included in the discussion. Table 1 contains the results of the agglutinative tests and the culture findings of the animals killed 1 day after discontinuation of treatment. While the agglutinative titers of the controls ranged from 1:100 to 1:400, the sera of 12 of the treated animals were negative in a 1:25 dilution. Furthermore, the control guinea pigs yielded Brucella on cultures in each instance, whereas 4 of the animals treated from the day of infection, and 4 in which treatment was begun one week after infection were negative on cultures. Six of the remaining treated guinea pigs yielded Brucella from 1 or 2 tissues only, and in 5 animals Brucella was isolated from all 3 tissues. However, the Brucella colonies isolated on culture plates from the treated animals were much fewer in number than in the case of the untreated controls. In Table 2, are presented the data on the animals killed 8 days after cessation of treatment. The blood sera of six of the guinea pigs treated from the day of infection were negative in a 1:25 dilution when tested against Brucella antigen. The tissues of 3 of these were negative on cultures, and 2 additional animals yielded only 1 and 2 Brucella colonies, respectively. All the guinea pigs in which treatment was begun 1 week after infection, and all the controls were positive to the agglutinative test and on cultures.

EXPERIMENT 2. Since the quantities of streptomycin used in the first experiment, namely, 5000 units daily, did not produce visible toxic effects, another group of animals was subjected to treatment with larger doses of this antibiotic substance.

Ninety-three guinea pigs were each injected subcutaneously with 2840 *B. abortus* of the same anaërobic strain as was used

in the first experiment. One week after infection treatment was begun on 40 guinea pigs, and the other 53 animals were left as controls. Throughout this experiment the treated guinea pigs received 20,000 units of streptomycin daily divided into 6 equal doses, and the injections were made subcutaneously at 3-hour intervals beginning at 9 A.M. and ending at 12 midnight each day.

Toxicity. No general toxic effects were noticed as a result of the injections of streptomycin. Toward the latter part of the experiment, however, thickening of the skin and subcutaneous tissue was observed in 6 animals in the region in which most of the injections were made, namely, the ventral side of the thorax and abdomen, and at autopsy these animals showed adhesions between the cutis and subcutis.

TABLE 1.—RESULTS OF AGGLUTINATIVE TESTS AND CULTURE FINDINGS ON GUINEA PIGS AUTOPSIED ONE DAY AFTER DISCONTINUATION OF TREATMENT

Treated animals*							Controls				
Guinea pig No.	Treatment begun	Length of treatment	Agglutinative titer at time of autopsy	Culture findings I.L.G.	L.	S.	Guinea pig No.	Agglutinative titer at time of autopsy	Culture findings I.L.G.	L.	S.
1	On day of infection	27 days	P 1:25	−	−	+	40	P 1:100	+	+	−+
2			Neg.	+	−	+	41	P 1:200	+	+	+
3			Neg.	−	−	−	42	P 1:100	+	+	+
4			Neg.	−	−	−	43	P 1:200	+	+	+
5			Neg.	−	−	−	44	P 1:100	+	+	+
6			P 1:50	+	+	−	45	P 1:200	+	+	+
7			Neg	−	−	−	46	P 1:200	+	+	+
8			Neg.	−	−	+	47	P 1:400	+	+	+
9			P 1:50	+	+	+	48	P 1:200	+	+	+
10			Neg.	−	−	−	49	+ 1:200	+	+	+
11			Neg.	−	−	+	50	P 1:200	+	+	+
12			Neg.	+	−	+	51	P 1:200	+	+	+
13	1 wk. after infection	20 days	P 1:50	+	+	+	52	P 1:400	+	+	+
14			Neg.	−	−	−	53	+ 1:200	+	+	+
15			P 1:50	+	+	+	54	P 1:400	+	+	+
16			Neg.	−	−	−	55	P 1:200	+	+	+
17			P 1:200	+	+	+	56	P 1:400	+	+	+
18			P 1:100	+	+	+	58	P 1:400	+	+	+
19			Neg.	−	−	−	59	+ 1:200	+	+	+

* Each animal received 5000 units streptomycin daily divided into 5 equal doses which were injected subcutaneously at 3 hour intervals between 9 A.M. and 9 P.M.

Agglutinative tests: Neg. = negative in 1:25 dilution; P = incomplete agglutination; + = complete agglutination.

Culture findings: I.L.G. = inguinal lymph gland; L = liver; S = spleen; + = positive; − = negative.

TABLE 2.—RESULTS OF AGGLUTINATIVE TESTS AND CULTURE FINDINGS ON GUINEA PIGS AUTOPSIED 8 DAYS AFTER DISCONTINUATION OF TREATMENT

Treated animals*							Controls				
Guinea pig No.	Treatment begun	Length of treatment	Agglutinative titer at time of autopsy	Culture findings I.L.G.	L.	S.	Guinea pig No.	Agglutinative titer at time of autopsy	Culture findings I.L.G.	L.	S.
20	On day of infection	27 days	Neg.	−	−	+†	60	P 1:100	+	+	+
21			P 1:25	+	+	+	61	P 1:800	+	+	+
22			P 1:50	+	+	+	62	P 1:200	+	+	+
23			Neg	−	−	+‡	63	P 1:200	+	+	+
24			P 1:25	+	+	+	64	P 1:100	+	+	+
25			P 1:25	+	+	+	65	P 1:400	+	+	+
26			Neg.	+	+	+	67	P 1:200	+	+	+
27			Neg.	−	−	−	68	P 1:200	+	+	+
28			Neg.	−	−	−	69	P 1:50	+	+	+
29			Neg.	−	−	−	70	P 1:400	+	+	+
30	1 wk. after infection	20 days	P 1:400	+	+	+	71	P 1:200	+	+	+
31			P 1:50	+	+	+	73	P 1:200	+	+	+
32			P 1:100	+	+	+	74	P 1:400	+	+	+
33			P 1:100	+	+	+	75	+ 1:200	+	+	+
34			P 1:100	+	+	+	76	P 1:400	+	+	+
35			P 1:100	+	+	+	77	P 1:200	+	+	+
36			+ 1:800	+	+	+	78	P 1:100	+	+	+
37			P 1:100	+	+	+	79	P 1:400	+	+	+
38			P 1:50	+	+	+					
39			P 1:100	+	+	+					

* Each animal received 5000 units streptomycin daily divided into 5 equal doses which were injected subcutaneously at 3 hour intervals between 9 A.M. and 9 P.M.

† Only 1 colony present.

‡ Only 2 colonies present.

Agglutinative tests: Neg. = negative in 1:25 dilution; P = incomplete agglutination; + = complete agglutination.

Culture findings: I.L.G. = inguinal lymph glands; L = liver; S = spleen; + = positive; − = negative.

Hemoglobin determinations on the blood prior to autopsy of the animals showed an average of 12.71 gm. of hemoglobin per 100 ml. of blood in the treated groups, and 13.27 gm. per 100 ml. of blood in the controls.

continued, at which time 35 treated guinea pigs and 29 controls were still alive. Twenty-four hours after cessation of treatment blood was obtained for agglutinative tests and hemoglobin determinations from 17 treated animals and 11 controls, and

TABLE 3.—RESULTS OF AGGLUTINATIVE TESTS AND CULTURE FINDINGS ON GUINEA PIGS USED IN EXPERIMENT 2

Treated animals*						Controls		
Guinea pig No.	Treatment begun	Length of treatment	Autopsied after cessation of treatment	Agglutinative titer at time of autopsy	Culture findings	Guinea pig No.	Agglutinative titer at time of autopsy	Culture findings
81				Neg.	−	121	P 1:50	+
82				Neg.	−	122	P 1:50	+
83				Neg.	+	124	Neg.	+
84				Neg.	+†	127	P 1:100	+
85				Neg.	−	128	P 1:100	+
86				Neg.	−	129	P 1:200	+
87				Neg.	−	131	P 1:50	+
88			1 day	Neg.	−	134	P 1:100	+
89				P 1:25	+	138	P 1:25	+
90				Neg.	+	139	P 1:50	+
92				Neg.	−	141	+ 1:50	+
93				Neg.	−			
95				Neg.	−			
96				Neg.	−			
97				Neg.	−			
98				Neg.	−			
99				Neg.	−			
100	1 wk. after infection	24 days		Neg.	−	143	P 1:400	+
101				Neg.	−	146	P 1:400	+
102				P 1:50	+	147	P 1:200	+
104			8 days	Neg.	−	148	P 1:400	+
105				Neg.	−	149	P 1:100	+
106				P 1:50	+	151	P 1:400	+
107				Neg.	−	153	P 1:400	+
109				Neg.	−	154	P 1:400	+
110				Neg.	−	156	P 1:100	+
111				Neg.	−	159	P 1:400	+
112				Neg.	−	160	P 1:400	+
113				Neg.	−	161	P 1:400	+
114				Neg.	−	165	P 1:200	+
115			15 days	Neg.	−	166	P 1:100	+
117				Neg.	−	168	P 1:100	+
118				Neg.	−	169	P 1:200	+
119				Neg.	−	172	P 1:200	+
120				P 1:50	+	174	P 1:400	+

* Each animal received 20,000 units streptomycin daily divided into 6 equal doses which were injected subcutaneously at 3 hour intervals between 9 A.M. and 12 midnight.

† Only 1 colony isolated from all 3 tissues.

Agglutinative tests: Neg. = negative in 1:10 dilution; P = incomplete agglutination; + = complete agglutination.

Culture findings: − = negative; + = positive.

Results of Therapy. A protracted cold spell during the early part of the experiment favored a respiratory disorder in most of the guinea pigs, which resulted in the death of 5 treated guinea pigs and 25 controls. The fact that a much smaller percentage of treated guinea pigs succumbed would lead one to suspect that the streptomycin might have helped to keep respiratory infections down in this group of animals.

At the end of 24 days of treatment, which was 31 days after infection, the administration of streptomycin was discontinued, at which time 35 treated guinea pigs and 29 controls were still alive. Twenty-four hours after cessation of treatment blood was obtained for agglutinative tests and hemoglobin determinations from 17 treated animals and 11 controls, and then they were killed and their tissues cultured. Eight days after discontinuation of therapy 10 more treated and 10 control guinea pigs were bled and their tissues cultured, and finally 15 days after cessation of treatment the remaining 8 treated animals and 8 controls were bled and their tissues cultured. The technique for culturing the tissues at autopsy was the same as in Experiment 1.

The results of this experiment are presented in Table 3. Of the 17 treated guinea pigs sacrificed 1 day after cessation of treatment, 4 animals yielded *B. abortus* on

cultures, and only 1 of these had detectable agglutinins in a 1:25 dilution of the blood serum. In the group of 10 treated guinea pigs autopsied 8 days after discontinuation of therapy 2 animals were positive on cultures, and had slight agglutinative titers; in the group of 8 treated guinea pigs autopsied 15 days after treatment was discontinued only 1 animal had a slight agglutinative titer and yielded *B. abortus* on cultures. On the other hand, all the controls were positive and *B. abortus* was isolated from all 3 tissues cultured. These results would indicate that contrary to the findings in the first experiment the percentage of treated animals which was positive to the agglutinative test and on cultures was not augmented with an increase in the interval after the time of cessation of treatment. Thus it would appear that the larger doses of streptomycin used in this experiment destroyed the Brucella organisms in most of the animals, and their tissues remained sterile when cultured 15 days after therapy was discontinued. These findings assume additional significance in view of the fact that the treatment was not begun until 1 week after infection, by which time the organisms had ample time to multiply and disseminate throughout the body. The 2840 Brucella cells injected into each animal represented at least 200 minimal infective doses because a guinea pig injected at the same time with 14 organisms, according to plate counts, was positive on cultures when autopsied 4 weeks after infection.

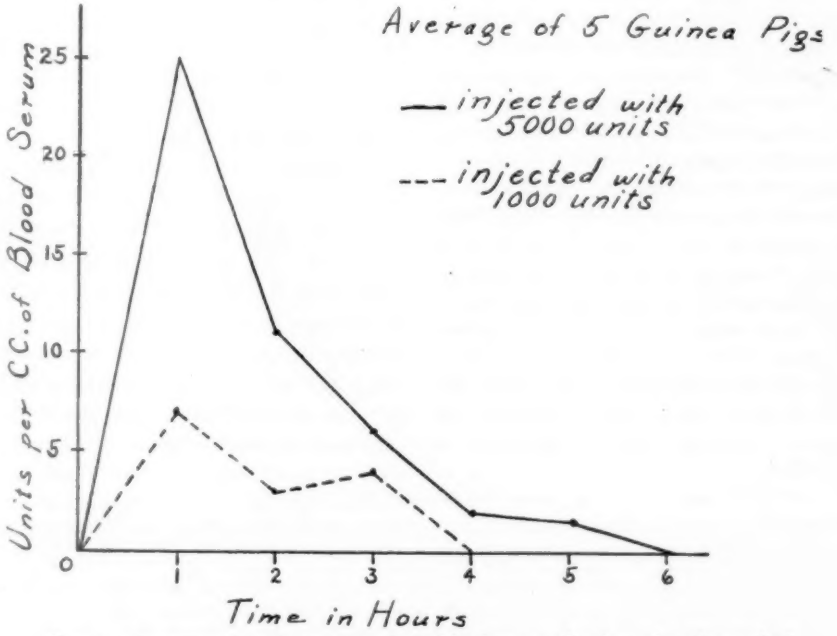

FIG. 1.—Concentration of streptomycin in blood serum of guinea pigs after a single subcutaneous injection.

Concentration of Streptomycin in the Blood Serum Following a Single Subcutaneous Dose. Two groups of 5 guinea pigs each were used to determine the concentration of streptomycin in the blood serum following a single subcutaneous injection of the drug. In one group, each animal received 5000 units of streptomycin while in the other each animal was injected with 1000 units of streptomycin. The animals were bled by cardiac puncture at hourly intervals for 6 hours, and the concentration of streptomycin in the blood sera was de-

termined by the plate cup method described by Stebbins and Robinson.[8] As indicated in Figure 1 the average concentration of streptomycin in the blood sera of each group reached its peak after 1 hour, being 25 units per cc. of blood serum in the group injected with 5000 units, and 7 units per cc. of blood serum in the group injected with 1000 units. Then the drug concentration decreased, and no streptomycin could be detected after 4 hours in the blood sera of the animals injected with 1000 units, and none could be detected after 6 hours in the blood sera of the animals injected with 5000 units.

Summary. Treatment of guinea pigs with 5000 units of streptomycin daily, divided into 5 doses, had a definite bacteriostatic effect upon *B. abortus*.

Four of 9 animals in which treatment was begun on the day of infection and continued for 27 days, and 4 of 10 animals in which treatment was begun 1 week after infection and continued for 20 days, were negative on cultures when autopsied 1 day after cessation of treatment. Of the guinea pigs which were cultured 8 days after discontinuation of therapy, 3 animals from the group of 10 in which treatment was begun on the day of infection were still negative while all the guinea pigs in which treatment was begun 1 week after infection yielded *B. abortus*.

Treatment of guinea pigs with 20,000 units of streptomycin daily, divided into 6 doses, seemed to eliminate the infection from most of the animals. The treatment was begun 1 week after infection, and was continued for 24 days. Of 17 animals autopsied 1 day after cessation of therapy, 4 were positive on cultures; of 10 animals autopsied 8 days after cessation of treatment, 2 were positive on cultures, and of 8 guinea pigs autopsied 15 days after discontinuation of therapy only 1 animal yielded Brucella on cultures.

Following a single subcutaneous injection of streptomycin, the blood serum levels are highest soon after injection of the drug. In guinea pigs injected with 1000 units no detectable concentration of streptomycin was found in the blood serum after 4 hours, and in those injected with 5000 units no detectable concentration of streptomycin was found in the blood serum after 6 hours.

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THE TREATMENT OF ACUTE RHEUMATIC FEVER WITH LARGE DOSES OF SODIUM SALICYLATE

WITH SPECIAL REFERENCE TO DOSE MANAGEMENT AND TOXIC MANIFESTATIONS*

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THE use of large doses of sodium salicylate in treating acute rheumatic fever has received new impetus since the report of Coburn[4] of the favorable results observed with this form of therapy. Coburn has postulated that with sufficiently high plasma salicylate levels, the sterile rheumatic inflammatory process can be suppressed and subsequent permanent valvular heart damage can be prevented.

We have attempted to evaluate our results with this method of treatment in 18 adults and 8 children with acute rheumatic fever. A variety of interesting metabolic and toxic reactions attributable to salicylate medication, were observed in our patients. Seven persons with diseases other than acute rheumatic fever were given large doses of salicylates for many weeks to examine further the effects of this drug.

Coburn stated that for effective therapy in rheumatic fever, the plasma salicylate level must be maintained above 350 μg. per cc. He has recommended 10 gm. of sodium salicylate and 6 gm. of sodium bicarbonate daily in divided doses for oral medication. During the first 2 weeks of treatment, he preferred to use intravenous medication in the form on a single daily infusion of 10 gm. of the drug or rarely 20 gm. in two separate infusions. The drug was then administered orally. In his group of patients receiving intravenous and subsequently oral medication as well as in the group receiving only oral salicylates, the observed clinical results were excellent.

Material and Results. ADULT GROUP. Our adult group consisted of 10 males and 8 females whose ages ranged from 16 to 46 years. Six were experiencing their first attack of rheumatic fever, 3 others, their second attack and the remaining 9 had had at least 3 episodes prior to the present one. In 11 of the 18 patients, there was a history of an upper respiratory infection preceding the present illness. Thirteen patients had swollen tender joints while 4 others had arthralgia. Fever at the time of admission, was present in 16 cases. In only 5 patients was there a family history of rheumatic fever.

In 5 of the 6 cases with presumably initial attacks of rheumatic fever, there was evidence of cardiac involvement as indicated by apical systolic murmurs in 3 cases, pericardial friction rubs in 2 cases, and abnormal electrocardiographic findings in 5 instances. All 12 patients with recurrent rheumatic attacks, had evidence of structural injury to the mitral valve or to both aortic and mitral valves. Other manifestations of cardiac injury in the 12 patients included cardiac enlargement (8 cases), signs of mild cardiac failure (6 cases), pericardial friction rubs (3 cases) and abnormal electrocardiographic findings (9 cases).

Examination of the blood at the time of entry disclosed significant anemia (volume of packed red cells 30 to 38 cc. %)

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in 11 of the 18 cases. Leukocytosis was present in 16 patients. The corrected sedimentation rate (Wintrobe method) was greatly elevated in all but one patient in whom it was normal. In this case, however, the leukocyte count was 16,000 per cmm.

In 11 patients, therapy with large doses of salicylates was initiated within 1 to 14 days after the onset of arthritis. In the other 7 patients, medication was not begun until 3 to 14 weeks after the onset of symptoms. In 12 patients, an intravenous infusion of 10 gm. of sodium salicylate was employed on the 1st day of therapy. In 5 patients, this was continued for 3 to 14 days and then oral medication was begun. In the other instances, the oral route was maintained throughout. In 17 patients, salicylate therapy was continued for 4 to 14 weeks while in 1 patient, death occurred from heart failure on the 8th day of medication.

During salicylate administration, clinical and laboratory evidences of rheumatic activity appeared to vanish in the 6 patients with initial attacks and in 5 of the 12 adults with recurrent episodes. In other patients there was usually some improvement but certain findings indicated that active infection persisted.

In every one of the 18 patients, rapid symptomatic improvement occurred within a few days after therapy was begun. In no case was there fever after the end of the 2d week. The pulse rate where it was elevated at the time of admission, usually returned to a normal range by the end of 2 weeks. Joint symptoms subsided within 3 to 6 days and in no case did they recur during therapy. In 8 patients, there was a marked improvement in nutritional status during the period of hospitalization as evidenced by a gain in weight ranging from 8 to 20 pounds (3.6 to 9.1 kg.). Two patients developed beta-hemolytic streptococcal pharyngitis during treatment; nevertheless there was no apparent activation of the rheumatic process.

Pericardial friction rubs which were audible in 5 of the cases at the time of admission, disappeared in 4 cases within 4 days and in 1 case, only after 2 weeks of medication. A decrease in intensity of cardiac murmurs was noted in the 3 patients with initial attacks in whom murmurs were present. Of the 12 patients with recurrent attacks, all of whom had murmurs prior to therapy, a decrease in intensity of cardiac murmurs during therapy occurred in only 2 cases. An abnormal prolongation of the P–R interval was observed in 7 patients initially and developed during therapy in two additional patients. In 5 patients this finding had disappeared within 30 days after beginning medication whereas it persisted in 4 patients during the entire period of treatment. In 4 additional patients there was inversion of T waves either in the first or second limb leads or in several precordial leads. This abnormality disappeared in 3 instances during therapy but in 1 case with pancarditis, persisted during $2\frac{1}{2}$ months of continued therapy during which high plasma salicylate levels were maintained.

Anemia, when present at the time of entry, disappeared in every instance during therapy. Leukocytosis observed initially in 17 patients, disappeared in 15 cases within 2 weeks while in 2 other patients it persisted throughout treatment. The corrected sedimentation rate (Wintrobe method) which was elevated in 17 patients, fell to normal in only 5 instances within 4 weeks after drug treatment was begun.

In 1 patient with an initial attack and in 5 patients with recurrent attacks, evidence of active rheumatic infection returned within 5 days after discontinuing therapy which had been maintained for 4 to 9 weeks. These patients, again, were given large doses of salicylates for a prolonged period with apparent improvement.

The follow-up period on these 18 patients has not been adequate to permit satisfactory evaluation of the results. One patient with an initial attack and 3 with recurrent attacks failed to return for study after leaving the hospital. One patient with recurrent rheumatic fever died on the

8th day of therapy. Another patient with recurrent acute rheumatic fever had an immediate recurrence of active infection after stopping salicylate medication on the 59th day of treatment. This patient died of salicylate intoxication 38 days after therapy was resumed.

The remaining 12 patients have been followed for periods of 2 to 20 months after termination of treatment. Nine have remained free from evidence of active infection. One additional patient in whom a good therapeutic response was obtained, developed another attack following a new respiratory infection which occurred 3 months after stopping medication. In 2 patients with recurrent rheumatic fever, both of whom had been given a second course of salicylate medication because of immediate recurrence following the first course, evidence of active rheumatic infection returned after the second course and persisted for many months thereafter.

The therapeutic response to large doses of salicylates in two adult cases of active rheumatic fever is described below.

Case Histories. CASE 1. O. T., a white male, 23 years of age, entered the hospital with an initial rheumatic attack of 4 days duration. This was characterized by the findings of fever, pharyngitis, arthritis, anemia, leukocytosis and an elevated sedimentation rate. The only abnormal electrocardiographic finding was an inverted T wave in Lead 4F.

The patient received 7.2 gm. (0.11 gm. per kg.) of sodium salicylate orally for 6 days. The daily oral dose was then increased to 12 gm. (0.18 gm. per kg.) and maintained at this amount for 31 days. There was rapid improvement with disappearance of clinical evidence of rheumatic activity. A systolic murmur which was noted over the apex of the heart during the second week of therapy, diminished in intensity during continued medication. An electrocardiogram taken 3 weeks after initiation of treatment was normal. The sedimentation rate and leukocyte count returned to normal values within 3 weeks. The volume of packed red cells was 33.5 cc. % on admission and 6 weeks later had risen to 45 cc. %. This patient has been followed for 18 months and has remained symptom free. The apical systolic murmur is no longer audible.

CASE 2. B. K., a white female, 22 years of age, entered the hospital because of migratory arthritis of 3½ months duration. There had been no previous attack. In the preceding 10 weeks, she had been hospitalized elsewhere and had received sodium salicylate in variable doses, sulfonamides and finally 2,000,000 units of penicillin. The patient had continued to have fever and swelling of joints and had had several episodes of severe precordial pain.

At the time of admission to our hospital, the pertinent findings included fever, swollen tender joints and an increased sedimentation rate. An electrocardiogram showed sharply inverted T waves in the precordial Leads V_1 and V_2.

The patient's oral salicylate dose was rapidly raised to 12.6 gm. (0.22 gm. per kg.) per day (Fig. 1, Case C). The plasma salicylate level was maintained between 350 and 450 μg. per cc. There was prompt symptomatic improvement. The sedimentation rate fell to a normal value after 6 weeks of treatment. The initial electrocardiographic changes disappeared by the 32d hospital day. On the 49th day, the patient acquired mumps and the sedimentation rate again became elevated. Salicylate medication was discontinued 5 days later. In the 4 months subsequent to treatment, this patient has shown no clinical evidence of rheumatic activity.

Clinical Material and Results. PEDIATRIC GROUP. Nine children, whose ages ranged from 9 to 15 years, received massive salicylate medication. Five were considered to be experiencing initial attacks. One of the 4 children with a history of previous rheumatic attacks, presented no clinical evidence of an active infection but was given large doses of salicylates in order to study the variations in plasma salicylate levels which may occur when constant dosage is maintained.

Arthritic manifestations were present in 4 of the 5 with initial attacks at the time salicylate therapy was instituted. All 5 had tachycardia and two showed electrocardiographic changes compatible with myocardial involvement. Four of this group were found to have moderately loud

apical systolic murmurs. Fever was present in two at the time of admission.

Two of the 3 patients with recurrent acute rheumatic attacks had joint symptoms. In addition, tachycardia and electrocardiographic changes were present but there were no murmurs or evidence of cardiac enlargement. In the 3d case, there was evidence of aortic and mitral valvular insufficiency. Fever was observed in only 1 of this group.

Laboratory studies revealed an anemia in 2 cases (volume of packed red cells 32 to 36 cc. %). Moderate leukocytosis was present in 4. The corrected sedimentation rate (Wintrobe) was elevated in 8 children with rheumatic activity.

Massive salicylate therapy was started within 10 days after the onset of rheumatic manifestations in 5 cases but rheumatic symptoms had been present at least 1 month in the remaining 3. Sodium salicylate was administered intravenously in doses of 0.10 to 0.33 gm. per kg. in 7 cases and orally in the 8th in a total daily dose of 0.22 gm. per kg. Intravenous medication was discontinued after 1 to 4 days and the same dosage was given orally in divided doses, every 4 hours day and night. With each oral salicylate dose, sodium bicarbonate in an amount ranging from 50 to 100% of the salicylate dose, was administered simultaneously. Enteric coated sodium salicylate tablets were not employed.

Symptomatic relief was obtained within 2 to 7 days. In 1 case the joint manifestations recurred on the 7th day after discontinuation of medication. Cardiac murmurs of great intensity, which were initially present in 3 instances, persisted without regression throughout and subsequent to salicylate therapy. The slight or moderate murmurs disappeared in 3 patients in whom they were present. Cardiac enlargement did not decrease in the 3 in whom it was demonstrated prior to the institution of salicylate therapy. Tachycardia persisted 7 to 10 days except in 1 patient in whom it persisted for many months.

The anemia, which was present in 2 patients at the time of entry, persisted throughout the period of salicylate medication. Leukocytosis continued in 2 and disappeared in 2 within 2 weeks after beginning treatment. The erythrocyte sedimentation rate returned to normal within 18 days in only 3 instances. In 2, it remained elevated for 2 months after salicylate therapy was begun.

There has been a period of 2 to 12 months since the discontinuation of salicylates in these patients. One of the 5 treated during an initial attack still has evidence of rheumatic activity and presents clinical signs of mitral valvular insufficiency. The 3 patients with recurrent acute rheumatic attacks are making satisfactory physical progress and are apparently free from rheumatic activity. Those without apparent rheumatic activity are being maintained on sulfanilamide prophylactically.

The response to salicylate therapy in 1 child with acute rheumatic fever is presented below in the form of a case abstract.

Case History. CASE 9. J. F., a 14 year old white boy entered the hospital because of malaise and migratory polyarthritis of 9 days duration. There was no past history of rheumatic episodes. Systolic murmurs were present over the apex and pulmonary area. There was swelling, redness and heat of the right knee. His blood showed a slight leukocytosis and an increased sedimentation rate. An electrocardiogram disclosed a prolonged P–R interval. There was no roentgenographic evidence of cardiac enlargement.

Sodium salicylate was administered intravenously for 2 days and then orally for 29 days in doses ranging from 0.13 to 0.17 gm. per kg. The plasma salicylate levels ranged from 330 to 412 μg. per cc. The sedimentation rate became normal by the 19th day while other evidences of active infection disappeared more rapidly. One week after sodium salicylate was discontinued, there was a recurrence of joint pain and swelling. The sedimentation rate increased and the P–R interval became prolonged again. On the 5th day after recurrence, salicylate therapy was resumed and maintained for 75 days. The plasma salicylate levels varied between 300 and 400 μg. per cc. The symp-

toms rapidly subsided. The sedimentation rate returned to a normal range on the 27th day. Six months later, the patient showed no evidence of rheumatic activity or of residual cardiac damage. He had made satisfactory physical progress, gaining 17 units on the Wetzel Grid[20] since his admission to the hospital. He had continued in the B–1 channel.

Salicylate Levels of the Plasma. In 19 patients with acute rheumatic fever and in 6 patients with other diseases, 6 to 30 plasma salicylate levels (average of 11 per person) were determined during the course of oral salicylate therapy. Other cases in which only a few levels were obtained, are not included in the discussion below. The plasma salicylate levels were determined according to a slight modification of the method of Brodie, Udenfriend and Coburn.[1] The error with this method as evidenced from many duplicate determinations and from recovery of known amounts of salicylate added to plasma, does not exceed 5%. All values are expressed in terms of plasma salicylic acid.

With oral therapy, all drug was administered at 4-hour intervals throughout the day and night. In 16 adults (10 with acute rheumatic fever and 6 with other diseases), oral medication consisted only of enteric coated sodium salicylate tablets. In 3 other adults, equal amounts of sodium bicarbonate were administered simultaneously with the enteric coated salicylate tablets. In the 6 children, equal amounts of sodium bicarbonate and non-enteric coated sodium salicylate tablets were employed. With each type of administration, it was observed that individuals varied greatly in the amount of drug which was necessary to maintain the plasma levels in the range of 300 to 400 μg. per cc. The range of dosage and the average dosage needed to obtain such levels with various forms of administration and in various groups is tabulated below (Table 1). The doses below (expressed in gm. per kg. of body weight) are the maximal doses needed in any given individual to obtain suitable levels. Often smaller doses produce satisfactory levels early in therapy and subsequently the dosage must be increased if high plasma salicylate levels are to be maintained.

The accompanying table clearly indicates that with both enteric coated sodium salicylate tablets and non-enteric coated tablets, there is great individual variation in dose requirement to obtain high plasma salicylate levels. In adults who received only enteric coated sodium salicylate tablets, the average oral dose necessary to obtain high plasma salicylate levels appeared to be greater in the group with acute rheumatic fever without evidence of cardiac failure than in an equal number of patients in whom cardiac failure was present with the active infection. The small group of adults who received equal amounts of sodium bicarbonate with the enteric coated salicylate tablets, appeared to require slightly more salicylate than the group of patients with active infection but without cardiac failure, who received only the enteric coated tablets. Patients without rheumatic fever seemed to require less drug than those with this disease to obtain comparable plasma salicylate levels. The average oral dosage of salicylates in the children who received non-enteric coated sodium salicylate tablets and equal

TABLE 1.—FACTORS INFLUENCING THE DAILY AMOUNT OF ORAL SODIUM SALICYLATE NECESSARY FOR MAINTAINING PLASMA SALICYLATE LEVELS ABOVE 350 μG. PER CC.

Type of case	Number studied	Cardiac failure	Type of salicylate tablet	$NaHCO_3$ (in amounts equal to salicylate dose)	Range of dosage (gm./kg.)	Average dosage
Adults with acute rheumatic fever	5	none	enteric coated	none	0.14–0.24	0.21
Adults with acute rheumatic fever	3	none	enteric coated	yes	0.26–0.30	0.29
Adults with acute rheumatic fever	5	yes	enteric coated	none	0.06–0.28	0.16
Adults with other diseases	6	none	enteric coated	none	0.11–0.15	0.12
Children with acute rheumatic fever	6	none	non-enteric coated	yes	0.11–0.31	0.25

amounts of sodium bicarbonate, did not differ markedly from the dose requirement in the adults with acute rheumatic fever who received enteric coated sodium salicylate tablets.

During oral administration of a constant dosage of sodium salicylate to patients with acute rheumatic fever, it is a frequent finding to observe a progressive rise in the plasma salicylate level during the first 2 weeks of medication. After this time, the level tends to drop. In 18 of the 26 patients with acute rheumatic fever, it was necessary, after 2 or 3 weeks of medication, to increase by 10 to 50% the original salicylate dosage in order to maintain suitable levels (Fig. 1). In only 4 instances did the level continue to rise after the first 2 weeks of therapy when dosage was constant and here it was necessary to reduce the dosage slightly. No similar decline in level after 2 weeks of therapy on constant dosage was noted in 5 adults without acute rheumatic fever who received oral medication for prolonged periods. This finding in the patients with acute rheumatic fever suggests that they develop an increased rate of excretion or destruction of the drug during prolonged therapy. Less than 0.3 gm. of salicylate was present in a single 24-hour stool specimen of 2 adults who had been receiving 18 gm. of enteric coated sodium salicylate daily.

In spite of careful regulation of dosage, the plasma salicylate levels from day to day or week to week in a given individual (both adults and children), often varied as much as 100 μg. per cc. on constant dosage. This makes it difficult to maintain the plasma salicylate level above 350 μg. per cc. at all times. If one strives to avoid this by maintaining an average plasma level of 450 μg. per cc. one brings the patient uncomfortably close to the toxic range.

In 4 patients, a total of 5 or 6 determinations of plasma salicylate were made at 3-hour intervals in a single day during continued oral therapy. The variation in plasma salicylate levels throughout the

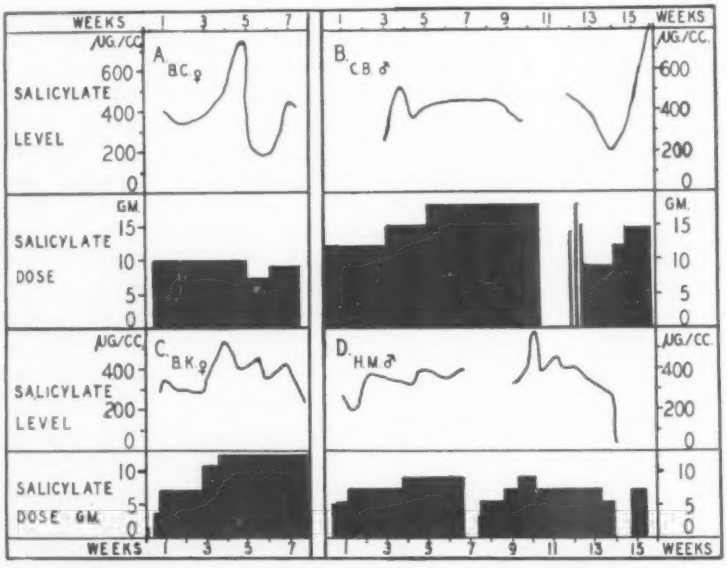

FIG. 1.—Relationship of plasma salicylate level to oral salicylate dosage in 4 patients.

Note in Cases B, C and D, the apparent reduction in plasma salicylate level which occurred on constant dosage and which necessitated increasing amounts of drug to maintain high plasma levels. Note, also, the unexplained rises in plasma salicylate levels on constant dosage in Case A and in Case B (during second period of drug therapy).

day varied from 50 to 90 μg. per cc. and the levels in each instance were higher in the morning than in the evening.

During oral salicylate therapy, while a constant dosage was being maintained, 2 adults (receiving enteric coated salicylate tablets) and 2 children (receiving non-enteric coated tablets) developed unexplained sudden increases in their plasma salicylate levels to values 200 to 300 μg. per cc. above previous levels (Fig. 1). The high levels were accompanied by toxic manifestations. The unexplained rise in level could not be attributed to significant variation in fluid intake or output in these patients.

Our experience with plasma salicylate levels attainable after intravenous medication is limited. In 3 adults who received 10 gm. of sodium salicylate intravenously, the plasma salicylate levels at the end of the infusion ranged from 400 to 600 μg. per cc. and 18 hours later they had fallen to levels varying from 250 to 350 μg. In 3 patients with mild cardiac failure and in 2 patients without cardiac failure, 5 or 6 plasma salicylate levels were obtained at approximately 3-hour intervals over the period ranging from 1 to 18 hours after completing a single infusion. The rate of decline of levels after terminating the infusion was considerably slower in the 3 patients with cardiac failure than in the 2 without failure. In 1 patient who received single daily intravenous infusions of 15 gm. of sodium salicylate over a 3-hour period, the volume of the infusion was maintained at 500 cc. for 1 week and at 1500 cc. for the next week while the time for the infusion was constant. The plasma salicylate levels which were determined daily, 18 hours after the previous infusion ranged from 400 to 540 μg. per cc. The observed fluctuations were not related to the volume of the infusion.

Salicylate Toxicity. A number of symptoms of salicylate toxicity have been observed in a total of 38 patients who have received large doses of this drug. This includes 27 patients with active rheumatic fever and 11 with other diseases. Therapy was maintained for 4 to 16 weeks in 30 patients and a shorter period in the remaining 8. The plasma salicylate level was determined at least once during each week of salicylate medication and usually 2 to 3 times a week.

In general, the severity of the toxic symptoms in a given individual was greater when the plasma salicylate level was high. No serious intoxication appeared in any patient whose plasma salicylate level was less than 400 μg. per cc. and often patients tolerated levels above 500 μg. for prolonged periods without difficulty. The highest plasma salicylate values ranged from 600 to 800 μg. per cc. in 6 cases, from 400 to 600 in 9 cases and from 200 to 400 in 13 cases.

Tinnitus which was usually accompanied by slight to moderate reduction in hearing was experienced by 34 of the 38 patients receiving salicylate therapy. In some instances, tinnitus did not appear until the plasma salicylate level exceeded 500 μg. per cc. whereas in others, it was noted when the plasma levels were below 200. Usually the severity of tinnitus in a given patient increased with rising plasma salicylate levels. Deafness to a noticeable degree, which was present in only 5 patients, invariably disappeared within 2 to 3 days after medication was stopped while tinnitus often persisted several days longer.

In 2 adults receiving oral salicylate medication, audiometer studies were made weekly during therapy and daily for the first 4 days after its discontinuance. The plasma salicylate levels in these individuals varied from 220 to 440 and 300 to 550 μg. per cc., respectively, during these studies. Reduction in hearing by air conduction below pretreatment values ranged from 20 to 35%. The more marked instances of loss of hearing seemed to occur when the plasma salicylate levels were highest. Following termination of medication, there was complete recovery of hearing in each patient in 3 to 4 days.

Nausea and vomiting which occurred in 25 of the patients receiving salicylate med-

ication, usually appeared early in therapy and later disappeared even though the salicylate dosage was subsequently increased and the plasma levels became higher. With very high plasma salicylate levels (above 500 μg. per cc.) nausea and vomiting were observed in 7 patients and disappeared when the dosage was reduced. These symptoms were likely to occur toward the end of the period of infusion in patients receiving intravenous medication.

During the course of prolonged salicylate therapy, an acne-form eruption appeared in 6 adult females and in 2 adult males. This usually became manifest during the 2d week and often persisted, although in one instance it disappeared during continued therapy.

Urinary changes during salicylate medication were infrequent. There was slight transient albuminuria in 4 of the 27 adults and one in the 11 children. In one severely intoxicated patient, albumin, red blood cells and granular casts appeared in the urine. It should be emphasized, however, that in at least 5 other patients with severe intoxication, no albuminuria occurred. Qualitative Benedict's tests on the urines frequently gave a trace to one plus reduction. In 4 adults and in 3 children, all of whom had plasma salicylate levels ranging from 400 to 600 μg. per cc., there was acetonuria lasting from 4 days to 3 weeks. In all patients, acetonuria was accompanied by a reduction in the plasma carbon dioxide combining power.

Hyperventilation was observed during salicylate therapy in 22 of the 27 adults and in at least 5 of the 11 children. It usually did not appear until the plasma salicylate level was above 400 μg. per cc. It was observed frequently in the period immediately following an intravenous infusion.

In all patients, careful search for a hemorrhagic tendency was made. In 14 patients, plasma prothrombin times were determined at least once a week and sometimes daily. The method of Quick[15] was used. It has been reported that sodium salicylate and other salicylate derivatives lengthen the prothrombin time.[7,13,16] We likewise have observed this change in patients receiving salicylates. This will be reported in detail elsewhere.[2] It deserves mention, however, that in spite of significant prolongation of the prothrombin time, hemorrhagic manifestations were observed in only one instance. This case was summarized below. In one patient of 5 in whom frequent tourniquet tests (Rumpel-Leede) were made during therapy, the test became positive for 4 days and then again became negative in spite of continued salicylate administration with maintenance of high plasma levels. In the other patients, the tourniquet test was negative.

No evidence of marked liver damage could be demonstrated in any patient during salicylate therapy. The icterus index, which was determined many times in each case, did not become elevated. The bromsulphalein test performed in 3 patients and the hippuric acid test in 1 patient during salicylate therapy, gave normal results. However, the cephalin flocculation test became positive in the 6 adults in whom this test was performed during therapy. These patients had plasma salicylate levels above 400 μg. per cc. The *in vitro* addition of sodium salicylate to serum from normal controls, failed to give a positive test.

Evidence of central nervous system disturbance was observed in a few patients, all of whom had plasma salicylate levels above 500 μg. per cc. This included hallucinations (5 cases), mental confusion (3 cases), vertigo (5 cases), diplopia (2 cases), "pounding of the head" (1 case), coarse tremor of hands (1 case) and coma (6 cases). Drowsiness and moderate euphoria were common manifestations at high plasma levels but were difficult to appraise.

Random determinations of plasma carbon dioxide combining power in 17 patients, demonstrated reductions to values ranging from 28 to 45 volumes % in 14 cases. No significant reduction occurred in any patient whose plasma salicylate level was below 400 μg. per cc. and the greatest reductions in the same patient and in the

entire group correlated roughly with the highest plasma salicylate values. The pH of venous blood (glass electrode method) performed in 2 patients with high plasma salicylate levels with reductions of their plasma carbon dioxide combining power below 40 volumes % but without acetonuria, were found to be normal.

A small number of determinations of blood urea nitrogen, plasma creatinine and plasma chloride revealed occasional slight rises which were inconstant in the same individual during therapy and inconstant for the group.

The corrected sedimentation rate showed surprising variation in its behaviour during massive salicylate therapy. In the adult group, the sedimentation rate returned to normal values in only 11 of the 18 patients with rheumatic fever after prolonged therapy, while in the 8 children, it ultimately returned to a normal range in every instance. In both adults and children it often took 5 to 8 weeks of continued medication before any significant reduction in the sedimentation rate was observed. More striking was the observation that, in the first 2 to 4 weeks after instituting salicylate medication, an increase in the sedimentation rate above the pretreatment levels occurred in approximately two-thirds of the patients. This rise was often marked and persisted usually less than 2 weeks but occasionally the sedimentation rate remained higher than the pretreatment levels for as long as 4 to 5 weeks of continued therapy. In 2 adults, this elevation persisted during many weeks of therapy and promptly fell to normal when therapy was stopped. Moreover in the instances where the sedimentation rate rose during the early period of therapy, it often afforded a striking contrast to the leukocyte count and other evidences of active rheumatic infection which were diminishing at this time. This suggested the possibility that the medication itself, might be influencing the sedimentation rate. In 4 of 5 adults with diseases other than rheumatic fever, a rise in the sedimentation rate likewise occurred after initiating salicylate therapy and, in 2 instances, return to pretreatment levels did not occur until therapy was discontinued.

If anemia be excluded, an elevation of the plasma fibrinogen is the most significant factor in producing an elevated sedimentation rate.[6,9,14] An increase in plasma globulin or lipid may also produce elevation of the sedimentation rate.[6,9] Other investigators[5,9] have demonstrated the correlation between plasma fibrinogen level and corrected sedimentation rate in cases of acute rheumatic fever. One of us (V. J., in conjunction with F. W. Clausen) has made multiple fibrinogen determinations in 5 adults with acute rheumatic fever prior to, during and following the course of salicylate therapy (Figs. 2 and 3). While there was no linear correlation, in every instance where the sedimentation rate (corrected or uncorrected for anemia) was increased, the plasma fibrinogen was elevated. Elsewhere one of us[3] has reported a similar rise in plasma fibrinogen and in sedimentation rates in control subjects with initially normal sedimentation rates and in rabbits that were receiving massive salicylate therapy. In many instances, the sedimentation rate and the plasma fibrinogen ultimately fall to normal values after the initial increase in spite of continued therapy and maintained high plasma salicylate levels.

Following discontinuation of therapy, a moderate increase in the sedimentation rate was observed in approximately half of the patients with rheumatic fever in whom the values had been normal prior to the discontinuance of medication. This rise usually appeared within 3 to 4 days, persisted 1 to 4 weeks and seldom was attended by other evidence of active infection. A similar type of response was noted after stopping treatment in cases of rheumatic fever by Ernstene[5] who used small doses of salicylates and by Coburn[4] who used massive doses.

The possibility that there is an increase in plasma volume during salicylate therapy was afforded by the finding of a marked drop in the volume of packed red cells

which occurred in 13 of 18 adults and in 2 of 9 children during treatment. This drop which ranged from 3 to 8% of the total packed cell volume, usually appeared within the first few days of treatment. With continued therapy, the hematocrit reading often returned to its original level or if anemia had been present, rose above the original value. In those patients exhibiting severe salicylate intoxication, a further drop in the volume of packed red

Case Histories. C. B. A 41 year old white, who had had repeated admissions to the hospital because of recurrent rheumatic attacks, pulmonary infections and mild congestive heart failure, was readmitted because of tender swollen joints of 10 days duration. On examination, the patient was found to have acute polyarthritis, mitral stenosis and insufficiency, auricular fibrillation and mild cardiac decompensation. There was moderate leukocytosis and an elevated sedimentation rate.

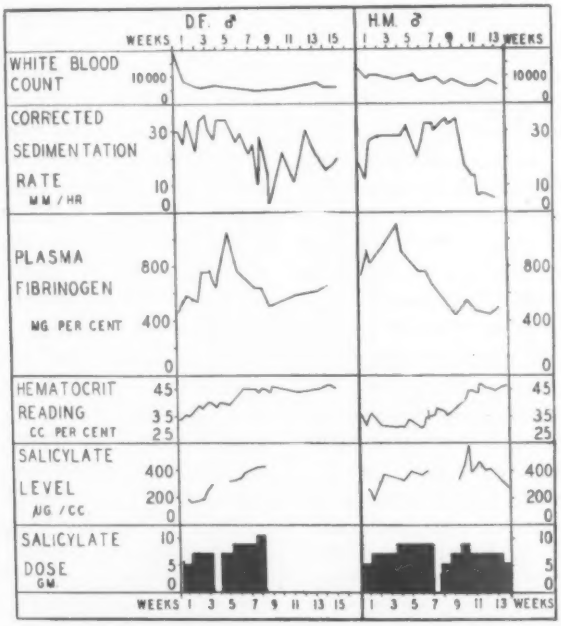

FIG. 2.—Changes observed in plasma fibrinogen, leukocyte count, sedimentation rate and hematocrit reading in 2 patients receiving large doses of sodium salicylate orally.

In Case D. F., observe the rise in fibrinogen and the rapid disappearance of anemia and leukocytosis during the first 6 weeks of therapy. In Case H. M., note again the initial increase in plasma fibrinogen with a subsequent decrease as well as the changes in the sedimentation rate and hematocrit reading.

cells occurred at such a time. Preliminary measurements by a dye method in several rabbits and in several persons receiving salicylate therapy also indicate that there is an increase in plasma volume during salicylate administration. Obviously, an increased plasma volume would be an unfavorable complication in a person with impaired cardiac function.

The salient features of 2 cases exhibiting severe salicylate intoxication are presented below.

The patient was digitalized and salicylate medication was begun (Fig. 1, Case B). For 71 consecutive days, he received equal quantities of sodium bicarbonate and sodium salicylate in maximal daily oral dosage of 18 gm. (0.29 gm. per kg.) of each. There was symptomatic improvement. However, on the 71st day, he suddenly developed pulmonary edema. Salicylate medication was discontinued for 1 week and then resumed. The day following cessation of medication, the joints again became swollen and tender. With renewed therapy, he received first 9

(0.14 gm. per kg.) and later 14.4 gm. (0.23 gm. per kg.) of sodium salicylate daily without added sodium bicarbonate. The plasma salicylate level 21 days after resumption of therapy was 432 μg. per cc. The volume of packed red cells was 46 cc. % at this time. Six days later, with the same dosage, the salicylate level of the plasma was found to be 812 μg. per cc. The patient was comatose and there was severe pulmonary congestion. Medication was discontinued. The previous 104.4° F. Clinically there was no evidence of a hemorrhagic tendency. Death appeared to have resulted from pulmonary edema.

Autopsy. Petechial hemorrhages were present in the epicardium and in the serosa of the small intestine. The brain appeared edematous and, on cut section, numerous petechial hemorrhages were present in the white substance. The heart was enlarged, weighing 700 gm. There was marked calcific stenosis of the mitral valve. Histo-

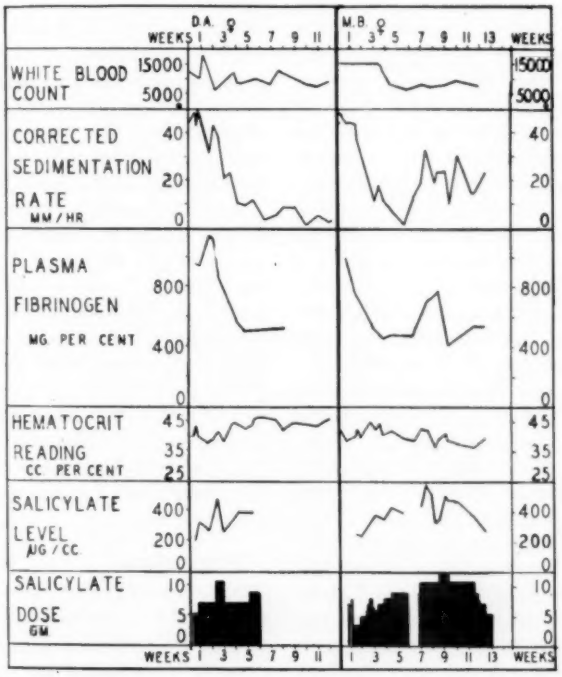

FIG. 3.—Changes observed in plasma fibrinogen, leukocyte count, sedimentation rate and hematocrit in 2 patients receiving large doses of sodium salicylate orally.

In Case D. A., note the changes in plasma fibrinogen and sedimentation rate during salicylate therapy. In Case M. B., observe the rise in plasma fibrinogen which occurred with readministration of the drug. Note also the fluctuations in plasma level as compared to variations in drug dosage.

day, the patient had been drowsy, had been hyperventilating and had complained of severe tinnitus and blurring of vision. With the high plasma salicylate level, the volume of packed red cells fell to 35 cc. % and the plasma prothrombin time became greatly prolonged. In spite of all supportive measures, the patient died 18 hours later. During the terminal period of coma, there was persistent marked cyanosis which was not relieved by the intratracheal administration of oxygen. Terminally the temperature rose to

logically the heart muscle contained many small foci of lymphocytes and histiocytes without characteristic Aschoff bodies. The lungs were congested and edematous. One small subpleural infarct was present. No significant gross or histologic abnormalities were observed in the aorta, kidneys, spleen, liver, adrenals, pancreas or striated muscle.

COMMENT. The petechial hemorrhages demonstrated in the brain, could have resulted from the prolonged period of

anoxia. A hemorrhagic diathesis, if it existed, was scarcely a significant factor in the fatal outcome. Death appeared to have resulted from pulmonary edema.

Case History. T. B. A 39 year old male moron with secondary syphilis was given sodium salicylate intravenously for 3 consecutive days to determine the effect of this drug on the prothrombin level. Prior to medication he was asymptomatic. On the first day he received 20 gm. of this drug (0.38 gm. per kg.) in 1000 cc. of saline. There was nausea and tinnitus following the injection. The next day (18 hours later) the plasma salicylate level was 336 μg. per cc. He was now given 15 gm. (0.29 gm. per kg.) of the drug. Nausea, vomiting and deafness appeared immediately after this injection and persisted for several hours. The 3d day, the plasma salicylate level prior to injection was 616 μg. per cc. He was given 10 gm. (0.19 gm. per kg.) of sodium salicylate. A few hours later he became delirious, developed marked dyspnea and bleeding from the nose and gums. He vomited frequently. The following morning, 18 hours after the last of the 3 injections, the plasma salicylate level was 564 μg. per cc. The patient was now comatose, dyspneic and cyanotic. There was pulsus alternans. Loud rhonchi were present in the chest. The 2d day after termination of medication, the patient was still stuporous and moderately cyanotic. The plasma salicylate level on this day was 318 μg. per cc. Bleeding from the nose and gums had ceased. The following day, he was less stuporous but was irrational and the speech was slurred. On the 4th day after the last salicylate injection, slight cyanosis was still present. Speech continued to be slurred. There were visual hallucinations. An inconstant divergent strabismus was noted. The patient recovered completely in the 2 subsequent days.

During the salicylate intoxication, interesting laboratory changes were observed. The volume of packed red cells, initially 42 cc. %, fell to 34 cc. % following the third injection and did not rise appreciably over 5 subsequent days and did not attain its original level for 3 more days. There was no rise in the icterus index. The prothrombin content of the plasma fell to 20% of the normal value on the 3d day of medication. The patient received 60 mg. of vitamin K intravenously at this time and 24 hours later, the prothrombin time was normal. The day following the last dose of salicylate, the carbon dioxide combining power of the plasma was 28 volumes % and 5 days later, it had returned to a normal value. At the peak of intoxication, the urine contained acetone, albumin and many granular casts. These changes disappeared during the 4 subsequent days. An electrocardiogram prior to therapy was normal. One taken 2 days after termination of medication showed depression of ST segments and lowering of T waves in the 3 standard limb leads. Serial precordial leads showed flattening and partial inversion of T waves. A tracing on the following day showed similar changes. However, the QT interval was now prolonged, being 0.52 second with a cardiac rate of 65. The expected QT for this rate is 0.35 to 0.45 second. Two days later, the QT interval was 0.42 second, the rate was 75 and T waves in the standard limb leads were flat. A few days later there were no electrocardiographic abnormalities.

Conclusions. Treatment with large doses of salicylates appeared to be effective in the 5 patients with initial attacks and in 4 of the 7 patients with recurrent attacks who could be followed for short periods after medication had been stopped. It is probable that the results would not appear so favorable if every patient could have been followed. Several of those who did not return for examination, had evidence suggestive of persistent rheumatic activity at the time of discharge from the hospital.

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EFFECT OF SODIUM BICARBONATE ON THE THERAPEUTIC EFFECTIVENESS OF SULFADIAZINE IN MICE

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Administration of sodium bicarbonate to rats fed sulfadiazine-containing diets has been shown to result in a marked depression of the sulfadiazine blood level.[8] Clinical studies[10] have also indicated some depression of blood level and increased urinary excretion of sulfadiazine after the oral administration of sodium bicarbonate. Since sodium bicarbonate has been shown to be effective clinically[4,5,6] and experimentally[7,8] in the prevention or correction of sulfadiazine-induced renal lesions, it became of interest to determine how sodium bicarbonate might affect the therapeutic activity of sulfadiazine.

Experimental. Purified diets were prepared in which sulfadiazine (0.1 to 0.5%) or sodium bicarbonate (0.3 to 4%) and sulfadiazine replaced equal weights of sucrose in the basal diet.* Female albino mice (N.I.H. strain) raised on Purina chow and weighing 17 to 22 gm. were fed one of the experimental diets. Groups of approximately 20 mice were used and 5 or more groups were compared in each experiment. The test organism was a virulent strain of Type 1 pneumococcus. When passed through mice every week or more often, this strain has maintained a virulence such that 0.5 cc. of a 6-hour culture in dextrose, veal-infusion broth at a dilution of 10^{-8} is uniformly lethal to mice when inoculated intraperitoneally. After the mice had been fed one of the experimental diets for 5 days, they were inoculated intraperitoneally with a 0.5 cc. of a 10^{-6} dilution of the culture containing approximately 500 organisms.† Inoculations were made in rotation, taking 1 mouse from each group under test. The mice were observed for a period of 2 weeks.

The data in Figure 1 indicate the mortality of mice fed diets containing varying amounts of sulfadiazine with and without sodium bicarbonate. The mortality was significantly greater among mice fed the diets containing 4% sodium bicarbonate. Thus the mortality was only 6% when mice were fed a 0.4% sulfadiazine-containing diet without sodium bicarbonate while it was 48% when sodium bicarbonate (4%) was incorporated in the diet. When administered in smaller amounts, the effects of sodium bicarbonate were less marked.

In order to determine the mechanism of action of sodium bicarbonate in increasing mortality, determinations were made of sulfadiazine intake (as calculated from food intake), absorption and blood concentration. Mice were fed a 0.3% sulfadiazine-containing diet (No. 1020) or a diet (No. 1032) containing 0.3% sulfadiazine and 4% sodium bicarbonate. Each diet was fed to 36 mice divided into 12 groups (cages) of 3. The average, daily food intake determined over a 4-day period was 7.8 gm. (range: 5.5 to 9.9) per 3 mice on diet No. 1020 and was also 7.8 gm. (range: 5 to 11.6) per 3 mice on diet No. 1032. Determination of the sulfadiazine content of feces collected during the 4 day period indicated that an average of 2.1% (range: 1.3 to 2.5) of the ingested sulfadiazine was unabsorbed in mice fed diet No. 1020 and 2.3% (range: 1 to 4) in mice fed diet No. 1032. Blood concentrations of sulfadiazine (free)‡ were determined by the Bratton and Marshall method[2] on blood samples obtained by decapitation and pooled for each group of 3 mice. As indicated in Figure 1, the average

* The basal diet (No. 1029) consisted of casein (Smaco) 18%, Crisco 8%, salt mixture No. 550[9] 4%, corn oil (containing 8000 units of vitamin A and 800 units of vitamin D [Natola] per gram) 2%, ethyl laurate (containing 100 mg. of α-tocopherol per gram) 0.12%, and cerelose 67.88%. Into 100 gm. of this diet were incorporated 1 mg. of thiamine hydrochloride, 1 mg. of pyridoxine hydrochloride, 4 mg. of calcium pantothenate, 2 mg. of niacin, 200 mg. of choline chloride, 0.001 mg. of biotin and 0.4 mg. of 2-methyl-1,4-naphthoquinone.

† We are indebted to Dr. Josephine M. Junge for providing the bacterial cultures used in these studies.

‡ Determination of "total" sulfadiazine revealed only traces or no detectable amounts of "conjugated" sulfadiazine.

blood sulfadiazine concentration was 13.2 mg.% (range: 10.3 to 17.3) for mice fed the 0.3% sulfadiazine-containing diet (No. 1020) and 7.1 mg.% (range: 6 to 8.5) for mice fed the 0.3% sulfadiazine, 4% sodium bicarbonate-containing diet (No. 1032). The average blood sulfadiazine concentration for 5 groups of 3 mice fed diet No. 1064 containing 0.5% sulfadiazine and 4% sodium bicarbonate was 10.7 mg.% (range: 9.2 to 12).

the result of increased renal clearance of sulfadiazine in the presence of sodium bicarbonate.

Of interest in relation to the present findings are the studies of Marshall and Litchfield,[9] and Rosenthal.[11] The former[9] found that sulfanilamide was more toxic to fasting mice than to fed mice and that this difference was related to the blood

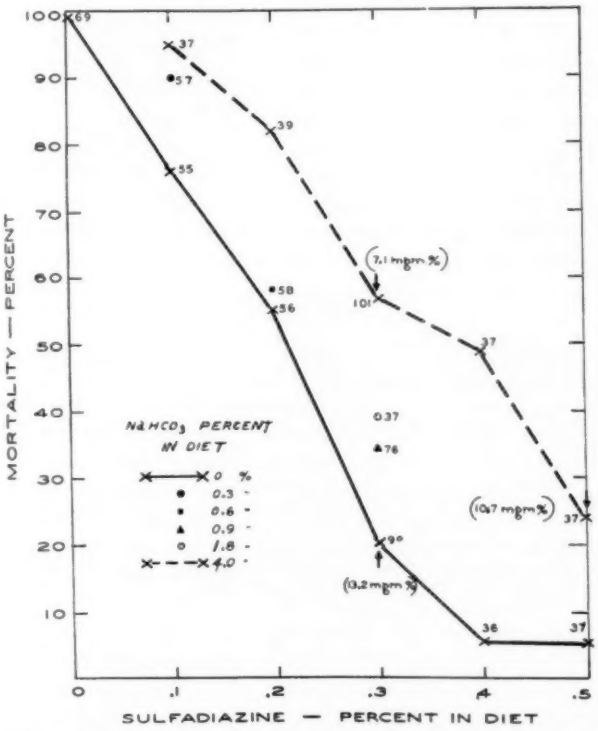

FIG. 1.—Mortality of mice inoculated with pneumococci and fed sulfadiazine-containing diets with and without sodium bicarbonate. The number at each point in the figure indicates the number of mice used to determine the % mortality at that point. The numbers in parentheses indicate blood sulfadiazine concentrations (see text).

Discussion. The inclusion of sodium bicarbonate in the diet has been found to impair the therapeutic effectiveness of sulfadiazine in mice. This sodium bicarbonate effect was not associated with any decrease in the intake or absorption of sulfadiazine but rather appeared to be related to a reduction of the blood concentration of sulfadiazine. The work of Beyer *et al.*,[1] and Earle[3] suggests that the depression of sulfadiazine blood levels is sulfanilamide concentrations. Rosenthal[11] showed that sulfanilamide was therapeutically more effective in mice on a low protein diet than in mice fed diets of higher protein content. Higher blood sulfanilamide concentrations were observed in the mice fed the low protein diet.

It is important to note that although sodium bicarbonate at dietary levels under 2% seemed to impair sulfadiazine therapeutic action, the most significant effects

were obtained with a level of 4% in the diet. This level of sodium bicarbonate represents approximately 10 times the weight of sulfadiazine when fed at a therapeutically effective level. For clinical use, Gilligan *et al.*[5] have recommended a sodium bicarbonate dose for adults of 18 gm. daily or approximately 3 times the weight of the usually accepted dose of sulfadiazine.

Clinical studies[4,5,6] and experimental work[7,8] have indicated the effectiveness of sodium bicarbonate in the prevention or correction of sulfadiazine renal lesions. We were able to show in rats[8] that severe renal lesions could be uniformly produced by sulfadiazine at a 1% level in the diet. These severe lesions were regularly prevented by sodium bicarbonate even when high blood sulfadiazine concentrations resulted from raising the dietary level of sulfadiazine to 4%. It would appear desirable to use sodium bicarbonate for the prevention of renal lesions but to give close attention to the maintenance of therapeutically effective blood levels of sulfadiazine during the administration of sodium bicarbonate.

Summary. Sodium bicarbonate in large amounts markedly depressed the blood concentrations and the therapeutic effectiveness of sulfadiazine in mice inoculated with pneumococci. These effects could be overcome by raising the dosage of sulfadiazine.

It is suggested that sodium bicarbonate be used to prevent renal lesions and that close attention be given to the maintenance of therapeutically effective blood levels of sulfadiazine.

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PENICILLIN IN ACUTE NEPHRITIS IN CHILDREN

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Following the experiments and research which have shown the antibacterial action of penicillin against a wide variety of pathogenic organisms, its use has now been extended to include a large number of different clinical diseases in man.

Acute nephritis is, in the majority of cases, secondary to some infective process. It has already been suggested that penicillin therapy is theoretically indicated in this condition, but we do not know of any report of a series of cases so treated.

In this small series of cases every patient was in a desperate condition, either due to neglect or due to repeated exacerbations within a short period in an anemic and devitalized child or the indiscriminate use of "sulfa" drugs based purely on blood counts showing a variable amount of leukocytosis. Even against these odds penicillin therapy carefully carried out has given extraordinarily encouraging results.

The essential feature of the disease being the recurrent nature of renal inflammation secondary to another infective focus, one postulates that if the primary focus can be eliminated the kidneys have a better chance of recovery and of remaining free from repeated attacks and consequent progressive degeneration. This primary infective focus, we know, is to be found in the nasopharynx, respiratory system or elsewhere. It is very often due to a hemolytic streptococcus, admittedly a highly susceptible organism to the "sulfa" group of drugs; but the kidneys do not tolerate this group well and the use of any form of intensive "sulfa" therapy in acute nephritis is fraught with grave dangers of toxic complications.

Evidence of renal toxicity has never been reported by workers who have used penicillin in the treatment of various diseases. The fact of its being completely devoid of toxic effects on the kidneys makes it increasingly imperative that a more extensive trial be given it in the treatment of acute nephritis, especially in those cases where an infective focus, demonstrable or otherwise, is the primary cause of the renal condition.

The absence of toxicity of penicillin for most other tissues is another great advantage in its use in acute nephritis. There is no toxic disturbance in the peripheral blood or in the hemopoietic system. Unlike the "sulfa" drugs, penicillin can be successfully used if there is severe anemia, marked leukopenia or even, as recorded, in the presence of agranulocytosis. I have seen in patients of pneumonia complicating typhoid, lowered leukocyte counts actually rise during or after penicillin therapy.

If pyrogen free penicillin is used, constitutional reactions such as fever, malaise, headache, etc., should not occur.

When the observations just mentioned are taken into account regarding the extremely low toxicity of penicillin, it becomes at once apparent that favorable results may be expected in its use in acute nephritis, a condition where the question of drug toxicity is one of utmost importance in view of impaired renal function. It is obvious that penicillin, or for that matter any other drug, can have no possible therapeutic effect on the damage already done to the renal cells. In those cases, however, where there is evidence of an active septic focus, acting as a strong bactericidal agent, it removes or eliminates the source of infection. Furthermore, it is conceivable that it removes the inflammatory congestion in the renal tissues, particularly the cellular exudate between the layers of Bowman's capsule and the exudative process all over the interstitial

TABLE 1.—ANALYSIS OF CASES

Case No.	Age	Sex	Days since onset	Daily range of temp. at start of treatment (° F.)	White cell total count (thous.)	Edema	Daily urine output at start of treatment (ounces)	Penicillin			Results	Ultimate results and comments
								Dosage (units, thous.)	Days treated	Total dosage (units, thous.)		
1	6 yrs.	F	12	102–104	12.7	Moderate	12	10, 4 hourly	3½	22.0	Satisfactory	Temp. came to nor. in 5 days but rose to 99° occ.; edema disappeared in 10 days; urine 20 oz. daily; albumin (tr.) remains
2	2 yrs. 8 mos.	M	37 (2nd ac. attack in 3 mos.)	99.6–103	15.0	Severe gen.; anasarca	8 hematuria	5, 3 hourly	8	640.0	Doubtful	Tonsils enlarged and inflamed; temp. remained 98 to 99° daily for 1 mo. before coming to nor.; edema diminished slowly but some remained on feet; alb., casts and r.b.c; persisted urine; general condition better
3	1 yr. 2 mos.	M	4	103–104	19.2	Moderate	Anuria (12 hrs.)	Started with 10, after 8th dose reduced to 7, 3 hourly	3	199.0	Recovered	Temp. nor. in 3 days; urine commenced flowing in 6 hrs; in 12 days urine free from alb. and r.b.c., 20 oz. daily; edema gone in 4 days
4	2 yrs. 6 mos.	M	7	99–101	9.0	Severe	Anuria (10 hrs.)	10, 4 hourly	6	360.0	Recovered	Temp. 97 to 99° for 15 days, then nor.; urine freely passed after 10 hrs., in 8 days free from all abnormalities; edema disappeared in 15 days; general condition fair; anemia receiving treatment
5	7 yrs. 6 mos.	F	4	100–101.8	14.5	Severe	6–7	10, 3 hourly	4	32.0	Recovered	Temp. nor. in 3 days; urine increased to 24 oz. daily in 3 days; no alb., casts or r.b.c.; general condition very good
6	4 yrs. 3 mos.	M	12	99–104	7.6	Severe all over body	Oliguria; hematuria+	10, 3 hourly	2	160.0	Died	Very toxic; bronchopneum. rt. lung; was on sulfadiazine; died without any response to treatment
7	4 yrs. 8 mos.	F	7	100–101	12.5	Moderate	Oliguria, few drops each time	10, 3 hourly	3	24.0	Recovered	Temp. nor. in 2 days; urine flow good in 3 days; in 7 days free from all abnormalities
8	11 mos.	M	8 (3rd attack in 1 mo.)	100–103	14.7	Very severe all over body	4	Started with 10, after 8th dose reduced to 5, 3 hourly	4	200.0	Recovered	Acute pharyngitis present; temp. nor. in 4 days; urine output 25 oz. in 3 days; edema gradually disappeared in 22 days; urine normal
9	1 yr. 1 mo.	M	5	97–100	12.0	Ascites only over feet	8–10	7, 3 hourly	3	168.0	Recovered	Temp. nor. in 3 days; urine output impr. from 1st day, free from alb. and r.b.c. in 7 days; general condition excellent
10	3 yrs.	M	15	98–99.6	10.7	Severe	12	10, 4 hourly	7	420.0	Recovered	Pharyngitis and bronchitis present; temp. nor. in 6 days; urine contained alb. and some r.b.c. for 1 mo.; anemia severe, being treated
11	8 yrs.	F	7	99–102.6	9.5	Moderate	15	10, 3 hourly	5	400.0	Satisfactory	Chronic tonsillitis and adenoids present; temp. 99.4° daily; edema less; urine free, contains alb. (tr.) and some r.b.c.; awaiting tonsillectomy
12	1 yr. 7 mos.	F	14	100–102	15.5	Moderate	10–12	10, 4 hourly	5	300.0	Recovered	Bronchitis present; temp. nor. in 4 days; edema disappeared in 7 days; urine 30 oz. daily, free from alb. and r.b.c. after 15 days; anemia severe, receiving treatment

kidney substance. This results in a virtual decompression of the nephrons, thus enabling more of these units numerically to function normally.

Choice of Cases. In this series, as stated before, 12 children with severe acute nephritis were treated. Almost all were in a critical condition and in some life was despaired of. In some cases evidence of a primary infective focus was demonstrable and in most the leukocyte count was raised. Rise in temperature was present in all. In some complete anuria or oliguria had set in with variable amount of edema. One child had severe hematuria, possibly aggravated by the administration of large doses of sulfadiazine.

Material and Method of Administration. Penicillin was supplied in rubber capped bottles containing 100,000 units in each in the form of a powder. This was dissolved in 10 cc. sterile normal saline and the solution was kept constantly in the refrigerator.

All injections in this series were given intramuscularly. All syringes and needles were sterilized in double distilled water and cooled before use.

The skin at the site of injection and the rubber cap of the bottle were cleaned with ether. The utmost care was taken to avoid leakage of the solution into edematous tissues around the site of injection.

Dosage. The dose per injection varied between 5000 and 10,000 units, administered at 3 hourly, or in some cases 4 hourly, intervals. The total dosage varied between 192,000 and 640,000 units, except in Case 6 which died while treatment was in progress.

Duration of Treatment. The number of consecutive days of treatment varied from 3 to 8. There is no doubt that the duration of treatment required depends to some extent on the length of the period before penicillin therapy is started. It is also evident that the improvement caused was continued long after the actual period of treatment.

Complications. No toxic phenomena or complications arose in any of the cases treated. In 1 case only (Case 11) the child complained of severe headache on the first day of treatment after the fifth injection. This was attributed to systemic or psychologic causes and was relieved by antispasmodics. Some of the older children became "needle shy" and some difficulty was experienced in continuing the treatment.

Results. In judging the results it is to be remembered that these cases were chosen for the very reason that they were *in extremis* and had failed to respond to any other form of treatment. There remains no doubt that penicillin has a definite place in the treatment of acute nephritis. In the cases which were not complicated by complete anuria but were nevertheless very serious due to the presence of oliguria, the response was remarkable. The temperature came down to normal in 36 to 72 hours and although in some it rose slightly above normal for a few days, the general improvement justified the stoppage of penicillin. The urine improved greatly in output and became free from albumin, casts and red blood cells. In Cases 1, 2 and 11 some abnormality remained in the urine after the end of treatment, but they became afebrile and the edema gradually decreased due to improved urine output. Case 6 was very toxic when treatment was commenced. This was the fourth acute exacerbation within 3 months and during the 12 days previous to this treatment large doses of sulfadiazine had been administered. For 2 days he had hematuria with only a few drops passed at a time. He died after 2 days treatment.

It may be noted that the word "recovered" is used in the table of cases to imply complete absence of symptoms and signs; *e. g.*, rise of temperature, edema, presence of casts, albumin and blood cells in the urine. It does not necessarily mean a permanent eradication of disease.

Summary. Twelve cases of acute nephritis in children have been treated with penicillin, all being of very severe type. There was 1 death after 2 days treatment; the results were satisfactory in the other 11.

The bactericidal action of penicillin is

employed to eliminate the causal infective focus.

Results, as judged by the rapid amelioration of signs and symptoms are encouraging, and a wider trial is recommended.

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PENICILLIN THERAPY IN ULCERATIVE COLITIS*

A Preliminary Report

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This report is on the results of penicillin therapy in 5 cases of chronic ulcerative colitis. It is realized that this is a small series of cases upon which to base a report; also the subsequent follow-up is admittedly inadequate. However, the immediate beneficial results warrant this report in the hope that others may be encouraged to try a prolonged large scale evaluation of penicillin therapy in this disease.

The present literature considers penicillin ineffective in chronic ulcerative colitis.[1,7] It is worthwhile considering the literature in regard to the penicillin dosage used in this disease as well as in certain other diseases. Infections due to nonhemolytic streptococci treated with penicillin are almost entirely in cases of subacute bacterial endocarditis. Early attempts to treat this disease with penicillin were unsatisfactory because only small and inadequate doses could be given.[9] More recent experience, using of 200,000 to 300,000 units a day for 2 to 3 weeks, has indicated at least temporary arrest of the infection in more than 50% of the cases.[5,10,12] Other infections such as primary syphilis appear amenable to penicillin therapy only in relatively high dosage. In reviewing the literature[7] it was found that the maximum dose used in ulcerative colitis was 840,000 units of penicillin in a period of 6 days, the average being about 140,000 units per day. It seemed reasonable to try a higher dosage in some cases of ulcerative colitis; a total dosage of 1,200,000 units in 5 days was arbitrarily chosen. The intramuscular route of administration was adopted, as it is fully as effective as the intravenous.[8] It is regretted that laboratory facilities to determine penicillin blood levels were not available. However, in order to establish a sufficiently prolonged and adequate blood level, the dosage was given every 3 hours.[1,8] Thus, 30,000 units were given intramuscularly every 3 hours up to a total of 1,200,000 units.

We do not infer that the pencillin therapy in these cases was a specific treatment for a disease due to a specific organism. It was simply an attempt to evaluate its effect in cases of chronic ulcerative colitis. The examinations of these patients did not reveal any indication of specific diseases such as bacillary dysentery, amebic colitis, tuberculous colitis, lymphopathia venereum, histoplasma capsulatum infection, or a deficiency syndrome such as sprue. The findings in each case indicated the so-called non-specific or idiopathic colitis. The proctoscopic findings were characteristic of the "streptococcic ulcerative colitis" described by Bargen[2] or the stage 2 or stage 3 of the acute stage of ulcerative colitis as classified by Bockus.[3] The lesions began in the most distal segment of the rectum, diffusely involved the entire bowel wall, and showed the characteristic hyperemic, edematous, friable, granular mucosa with scattered areas of ulcerations. It is believed that the colon roentgen-ray findings were not necessary for the diagnosis but did serve in the estimation of the extent and severity of the disease.

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Case Abstracts. Case 1. A white male, 34 years, stated that about 1935 he noticed the onset of severe pains in the umbilical area with radiation to the splenic region associated with alternating attacks of diarrhea and constipation. The color of his stools varied from a light brown to red to black. He was treated at that time by a private physician for colitis. These symptoms occurred intermittently throughout the years. In June, 1943, he developed severe peri-umbilical pains accompanied by approximately 20 bowel movements daily. Gross blood was present in the stools at intervals. Hospitalization was required for a period of 1 week. The diarrhea continued consisting of 5 soft light brown stools a day. The patient was rehospitalized elsewhere on four different occasions. Each time he had complained of 15 to 20 stools daily frequently containing blood. On admission he was having 5 to 10 liquid and soft stools daily usually associated with fresh blood. The intermittent peri-umbilical pain had continued.

The physical examination was essentially negative except for slight tenderness below the umbilicus on abdominal pressure. The red cell count was 4,000,000 with 10 gm. of hemoglobin. The white cell count was 15,200 (77% neutrophils). Repeated stool examinations for parasites and pathogenic organisms were negative. The proctosigmoidoscope was passed 8 inches revealing multiple areas of ulceration throughout. The ulcers were more profuse in the rectum and averaged about 6 mm. in diameter. The mucosa was very hyperemic with occasional areas of pin-point hemorrhage. Mucosal edema and friability were marked. The barium enema revealed redundancy of the sigmoid and descending colon with some lack of haustration; after emptying there was a small amount of barium throughout the colon and a rather stringy appearance suggesting spasticity. There was slight irregularity in the haustration of the distal half of the transverse colon.

The patient was given a high protein, high carbohydrate, low residue diet, multi-vitamins, and ferrous sulfate. Proctosigmoidoscopy was done on the 3d, 10th, and 17th hospital days. The only change noted was a slight decrease in the friability of the mucosa. The 5 day course of penicillin was then given. On the 25th hospital day, 3 days after penicillin was stopped, proctosigmoidoscopy revealed only slight hyperemia and granularity of the mucosa. The ulcerations and friability previously observed had completely disappeared. On the 43d hospital day, examination revealed no recurrence of the rectal ulcers. One month later, the patient wrote that he was having well-formed stools and he felt greatly improved.

Case 2. A white, male, 32 years, had noticed since 1935 recurrent episodes of mid-abdominal colic accompanied by watery stools which at times were associated with fresh and old blood. In January, 1944 he had been hospitalized for about 10 days for these symptoms. On admission he was having 5 to 6 stools daily. He described them as watery, yellow, and occasionally red or black. He also complained of a constant tenesmus. He had lost 5 pounds of weight in the past year.

The physical examination was essentially negative except for slight tenderness on pressure over the left lower abdominal quadrant. The urinalysis, complete blood count, and sedimentation rate were within normal limits. Repeated stool examinations for parasites and pathogenic organisms were negative. Proctosigmoidoscopy for a distance of 10 inches revealed numerous ulcerations averaging about 8 mm. in diameter. They were most marked in the caudal half of the rectum. There were at least 15 ulcerative areas, their base being grey or hemorrhagic in type. The mucosa was hyperemic and edematous. The barium enema was essentially negative.

The patient was given a high protein, high carbohydrate, low residue diet, multi-vitamins, and ferrous sulfate. He was placed at bed rest and given an 8 day course of sulfadiazine with an initial dose of 4 gm. and then 1 gm. every 4 hours. A blood "sulfa" level of 10 to 12 mg. was obtained. On the 19th hospital day, 2 days after sulfadiazine was discontinued, proctosigmoidoscopy was repeated. The numerous ulcerations and hyperemia were still present. The following day he was started on the 5 day course of penicillin. Two days after its completion, proctosigmoidoscopy revealed the disappearance of the ulcerations except for 3 small areas which were covered by a superficial thrombus and were apparently healing. The mucosal hyperemia and edema had also disappeared. Examination 5 days later showed no evidence of ulceration. Proctosigmoid-

oscopy 1 month and 2 months later revealed no recurrence of the ulcerations. The patient stated that he felt greatly improved. He was having only 1 or 2 well-formed stools daily with no evidence of bleeding.

CASE 3. A white male, 36 years, while in North Africa in May 1943, developed diarrhea consisting of 15 to 20 watery stools daily some of which contained blood. This persisted for 3 weeks and soon afterward recurred for a period of 1 week. He was treated with oral bismuth preparations and improvement resulted. After feeling well for 2 months, the symptoms recurred. He had 10 to 15 watery bloody stools daily associated with lower abdominal cramping pains. From August 1943 to the latter part of 1944 while in Italy he continued to have frequent loose stools which were sometimes bloody and were associated with abdominal cramps and generalized weakness. Treatment was symptomatic in type. On January 29, 1945, shortly after his return to this country he was hospitalized on our service. He complained of diarrhea averaging 8 liquid or soft stools daily often associated with fresh blood. Lower abdominal cramps were also present.

The general physical examination was essentially negative. The urinalysis and complete blood counts were within normal limits. Repeated stool examinations for parasites and pathogenic organisms were negative. The barium enema Roentgen ray was negative. Proctosigmoidoscopy revealed large external hemorrhoids encircling the anal orifice. The instrument was passed with great difficulty for a distance of only 7½ inches because of the patient's discomfort. The mucosa was extremely redundant, edematous and hyperemic. There were multiple ulcerations averaging about 6 mm. in diameter throughout the area of visualization. The ulcers contains a sero-sanguineous exudate.

He was treated with a high protein, high carbohydrate, low residue diet with multivitamin tablets, bismuth subcarbonate and ferrous sulfate for 2 weeks. By that time he was having about 4 soft stools daily associated with considerable flatus. However, proctosigmoidoscopy revealed essentially no change in the pathologic signs previously described. He was given an 8 day course of sulfadiazine with a blood level of about 10 to 12 mg. per 100 cc. Three days after its completion proctosigmoidoscopy showed no apparent change in the ulcerations but the mucosa was somewhat less hyperemic and edematous. There was essentially no change in his symptoms. A 10 day course of chiniofon 0.25 gm. t.i.d. was given. At its completion, proctosigmoidoscopy showed no change in the ulcerations and his symptoms remained the same. On his 38th hospital day he was started on the 5 day course of penicillin. On the 45th hospital day, examination revealed apparent healing of all the ulcerations and an essentially normal mucosa except for granularity and redundancy. Proctosigmoidoscopy on the 52nd hospital day revealed the same findings. The stools were formed, there was no excessive flatus, and the rectal bleeding had ceased. Sixty days after the course of penicillin, examination revealed no recurrence of the rectal ulcerations.

CASE 4. A white male, 30 years, in March 1944 suddenly developed a diarrhea consisting of 5 liquid and soft stools daily with some blood streaking, pus and mucus. Two weeks after onset of his symptoms he was hospitalized elsewhere. He stated that a diagnosis of ulcerative colitis was made. During 6 months hospitalization, general therapy and sulfaguanidine resulted in improvement so that he was passing 2 formed stools daily with no gross blood, pus or mucus. After discharge from the hospital in October 1944 he felt well until December 1944 when the same symptoms recurred. He was rehospitalized elsewhere on February 10, 1945. The proctosigmoidoscopy was reported: "Shows multiple small punctate ulcers, bleeding areas surrounding submucosal hemorrhages, and petechiae in moderate numbers." A diagnosis had been made of "severe chronic ulcerative colitis with submucosal hemorrhages." Treatment consisted of a bland low residue diet, vitamin supplements, and sulfaguanidine 2 gm. 4 times daily from February 16 to March 1. He was transferred to this hospital on March 2, 1945.

The physical examination revealed mild, deep, diffuse abdominal tenderness. The edge of the liver was palpable at the costal margin and was slightly tender. He continued to have 3 or 4 liquid or semisolid stools daily with some fresh blood, pus and mucus. There was excessive flatus. Proctosigmoidoscopy (9 inches) revealed multiple ulcerations ranging up to 5 mm. in diameter and containing a seropurulent-hemorrhagic

exudate. There was diffuse hyperemia, numerous pin-point hemorrhages with some areas of larger bleeding, marked edema and increased friability. Using a platinum wire loop under direct visualization cultures were made directly from the ulcers. These were subsequently reported as *E. coli*. Repeated warm stool examinations were negative for parasites and the cultures were negative for pathogenic organisms. The Frei test was negative. The red cell count was 3,770,000 with 11 gm. of hemoglobin. The white cell count, the differential count, and the sedimentation rate were normal. The barium enema revealed an absence of haustral markings in the distal colon. There was no definite serration of the bowel wall and the mucosal pattern of the descending colon was not destroyed. On the 8th hospital day the 5 day course of penicillin was started. No other treatment was given except for the high protein, high carbohydrate, low residue diet. On the 15th hospital day, proctosigmoidoscopy showed apparent healing of all the previous ulcerations. However, multiple pin-point hemorrhagic areas were still present. The mucosa was not as friable and the edema had decreased. The same course of penicillin was repeated in addition to the administration of vitamin supplements, ferrous sulfate, and belladonna rectal suppositories. On the 22d hospital day, proctosigmoidoscopy (10 inches) revealed mild to moderate hyperemia and edema while the hemorrhagic areas were less marked and appeared to be subsiding. No ulcers were present. Mild sedatives, antispasmodics, bismuth subcarbonate, and intramuscular liver were given in addition to the other general therapy. Proctosigmoidoscopy was done at weekly intervals revealing gradual improvement so that on the 52nd hospital day there were only a few pin-point hemorrhages, mild to moderate hyperemia and friability and an increasing diffuse granular appearance of the mucosa. He was having usually 2 fairly well-formed stools daily with only an occasional small amount of blood or mucus. There was no tenesmus or abdominal pain. From the 60th to the 65th hospital days, chiniofon 0.25 gm. q.i.d. was given. It was discontinued then because of increasing diarrhea. Proctosigmoidoscopy on the 78th hospital day revealed definite improvement consisting of decreased hyperemia, edema, and friability, the absence of hemorrhagic areas, and a slightly granular appearance of the mucosa. There were no ulcerations. The final examination on the 85th hospital day revealed no recurrence of the ulcerations or hemorrhagic areas.

CASE 5. A Negro, 29 years, stated that he had noticed rectal bleeding for 2 years. He had frequent episodes of diarrhea consisting of 6 or 7 soft stools daily. The fresh blood usually discolored the stools and also followed the bowel movement. Tenesmus and abdominal cramps occurred frequently. He had lost 12 pounds of weight in 2 years. An accurate history was difficult to obtain from the patient.

The general physical examination was essentially negative. The red cell count was 3,950,000 with 11.5 gm. of hemoglobin. The white cell count was 7100 with a normal differential count. The serum protein was 7.6 gm. Repeated stool examinations were negative for parasites and pathogenic organisms but revealed large amounts of blood, pus and mucus. A Frei test was negative.

Proctosigmoidoscopy (10 inches) showed diffuse ulcerated, hemorrhagic areas throughout with a well marked polypoid, hyperplastic inflammation most prominent on an adjacent to the lower and middle rectal valves. Biopsies of two of the polypoid lesions revealed microscopically a heavy diffuse and focal round cell infiltration with numerous eosinophiles throughout the mucosa and subjacent fibrous and muscular tissues. The appearance indicated no specific type of histopathologic lesion and the diagnosis was chronic proctitis. The patient was afebrile but it was considered inadvisable to attempt a barium enema Roentgen ray because of the above findings and his general condition.

Treatment consisted of a high protein, high carbohydrate, low residue diet, phenobarbital, belladonna, ferrous sulfate and multivitamin tablets. An initial dose of 4 gm. of sulfadiazine and 1 gm. every 4 hours were given for 6 days. Three days later, on the 23d hospital day, proctosigmoidoscopy revealed essentially no change. Chiniofon 0.25 gm. t.i.d. was prescribed for 10 days. Again the examination was unchanged. The 5 day course of penicillin was given. On the day it was completed, proctosigmoidoscopy showed only a few remaining ulcerations on the valves of Houston and a marked decrease in the hyperplastic inflammation, friability,

edema and hyperemia. Examination 7 days later revealed no definite ulcerations although a few superficial erosions were present on the rectal valves. The proliferative granulations had decreased. A 2d course of penicillin was given in the increased dosage of 40,000 units every 3 hours for 10 days. Proctosigmoidoscopy then revealed a decrease in the degree and extent of the polypoid granulating areas and some local hyperemia and friability still persisted. No ulcerations were present. Symptomatically the patient was much improved. He had no cramps or tenesmus. He still had 2 to 4 soft stools daily with occasionally a little fresh blood. He had gained 14 pounds of weight since the institution of penicillin therapy.

Discussion. Chronic ulcerative colitis is a disease of unknown cause. The term probably embraces a group of variable colon and systemic signs and symptoms depending on how different individuals react to a single injurious agent or it involves a similar syndrome resulting from multiple etiologic agents. The course of the disease is extremely varied and unpredictable. Improvement both subjectively and objectively in response to a new form of therapy may be purely coincidental to the natural history of the disease in individual cases. It may be influenced by psychogenic factors incident to the hope of a new effective treatment. The enthusiasm of the physician as well as the patient may be of considerable effect in a partial or total psychosomatic disorder. Only on such a basis can any new type of therapy be properly evaluated.

At the present time there is no widely accepted specific treatment for this disease. Diet, sulfonamides, serums, vaccines, vitamins, and many other agents have been employed. There is no doubt that each has contributed to the improvement or possibly "cure" in many cases. Yet not one of these agents has been considered to have a specific curative effect in a significant number of instances. Many of these measures were used at various stages in conjunction with penicillin therapy in our patients. There seems little doubt that whatever benefit penicillin may exert it will be increased by other measures, especially those designed to correct the primary or secondary nutritional defects.

These patients were all considered to be moderate to severe cases as regards the extent and degree of the local disease. However, none were severe from the standpoint of systemic reaction. They did not represent either the acute severe fulminating septic type, nor the severe chronic continuous type with a narrowed, shortened fibrotic colon. It will be noted that one case was given preliminary dietary and nutritional therapy for 2 weeks without improvement. The 2d case was given iron and sulfadiazine in addition to nutritional therapy without appreciable benefit. The 3d and 5th cases were given preliminary sulfadiazine and chiniofon therapy without any change in the rectal ulcerations. The 4th case was treated without preliminary therapy (although he had sulfaguanidine, vitamins, and a low residue diet at another hospital) and his supportive therapy was given following the initial course of penicillin.

In the 5 cases there was disappearance of all ulcerations during or immediately following the administration of penicillin. This result was the striking feature of the penicillin therapy. The 4th case still had residual pin-point hemorrhages, slight edema, hyperemia, friability, and granularity (all these latter signs being greatly improved over the initial examination). The 5th case still had mild friability and hyperplastic granulations. Additional penicillin therapy did not seem to effect as rapid or possibly as complete disappearance of these pathologic signs. These cases were considerably improved symptomatically while the first 3 patients obtained complete relief. In our experience with these types of cases, the rapid improvement in the proctosigmoidoscopic findings has rarely if ever occurred under any former therapeutic regimen. The duration of follow-up is inadequate for any long term evaluation at this time. However, even the immediate beneficial effect is of considerable importance. It is hoped that a more complete

conclusive report may be published later. At the present time penicillin apparently produces at least a remission in some mild and moderately severe cases of chronic ulcerative colitis and effects an improvement in other moderately severe cases. Arrest of the inflammatory process is the maximal benefit conceivable in those having irreversible fibrotic changes in the bowel wall.

The explanation for the effectiveness of penicillin is not clear. Possibly it acts within the tissues of the bowel wall on one or more of the infective agents such as penicillin susceptible streptococci. It is the usual belief that within the lumen of the bowel penicillin is rapidly inactivated by the intestinal bacteria.[1] However, there is a recent report of penicillin administered in rectal suppositories resulting in sustained blood levels.[11] It does not follow necessarily that it would be effective as a local application in inflammatory rectosigmoid lesions, but through its systemic action may be effective by the rectal route. The optimum dosage is still unknown. As in subacute bacterial endocarditis higher dosage over a longer period than 5 days may increase its effectiveness in ulcerative colitis. We contemplate trying the administration of 40,000 units every 2 or 3 hours for 10 days in a few cases. Recent reports indicate that the more convenient oral route of administration may be practical under some conditions.[4,6]

Summary. Penicillin in dosages of 30,000 units intramuscularly every 3 hours for 5 days up to a total of 1,200,000 units was administered to 5 cases of chronic ulcerative colitis.

The disappearance of the rectal ulcerations in each case within 1 week after the completion of one course of penicillin was the prominent feature. Improvement also occurred in the other pathologic signs. Improvement in subjective symptoms and the character of the stool was marked in each instance.

The results in these few cases indicate that adequate penicillin therapy may be of considerable value in the treatment or control of some cases of chronic ulcerative colitis. A trial of high dosage penicillid therapy on a large scale with a prolongen case follow-up is believed justified.

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COMBINED PENICILLIN AND SULFADIAZINE THERAPY IN PNEUMOCOCCIC PNEUMONIA*

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Numerous reports have already established the value of penicillin in the treatment of pneumococcic infections.[6,8,9,11] It is the purpose of this study to evaluate further the effectiveness of penicillin in the treatment of pneumococcic pneumonia by comparing 2 series of cases similar in severity of the disease cared for in the same hospital by the same staff and treated with the same general therapeutic routine. The only variation in treatment was that severely ill patients in the first group received adjuvant type specific antipneumococcic serum, while for similar indications a comparable number in the second group received adjuvant penicillin.

In the period from September 1, 1943 to May 1, 1945, 1620 consecutive patients with pneumococcic pneumonia were treated at the Permanente Foundation Hospital. The diagnosis of pneumonia was substantiated in every case by indisputable physical findings and a positive chest roentgenogram. No case in which the diagnosis of pneumonia could be questioned was included in this series. Patients with pneumonia as a contributory diagnosis to another illness were excluded. Sputum examinations in every case showed the predominant organism to be the pneumococcus. Specific typing by Neufeld method was obtainable in 70% of cases.

Procedure. The general therapeutic regimen which was followed in this hospital for the treatment of pneumococcic pneumonia has been outlined elsewhere.[3] Every patient who was moderately or severely ill received maximum therapeutic doses of sulfadiazine, since it has been demonstrated[4] that the use of higher doses of sulfadiazine resulted in a marked decrease in mortality rate, a decrease in incidence of common complicating conditions, and a decrease in length of hospital stay, without any notable changes in incidence of drug toxicity. Hence, the majority of patients received an initial dose of 5 gm. of sodium sulfadiazine in 500 cc. of $\frac{1}{6}$ molar solution of sodium lactate intravenously followed by sufficient sulfadiazine administered orally to maintain average blood sulfadiazine concentrations from 12 to 20 mg. per 100 cc.[4] Adjuvant therapy in the form of fluids, expectorant cough mixtures, oxygen inhalation, and alkali (sodium bicarbonate, sodium lactate, or potassium bicarbonate as indicated) were used routinely.

Comparative Therapeutic Efficacy of Combined Penicillin and Sulfadiazine Therapy. From May 1, 1944 to May 1, 1945, 872 patients with pneumococcic pneumonia were treated as outlined above. Of these patients 195 (22.4%) were treated with penicillin in addition to sulfadiazine for indications presented below. In this group of 872 patients, 13 (1.5%) died (Table 1). All deaths occurred among the 195 severely ill patients who had received combined therapy.

In the previous 8 month period from September 1, 1943 to May 1, 1944, 748 patients with pneumococcic pneumonia were treated with the same general therapeutic regimen. The only differences in treatment were that (1) no penicillin was used, and (2) type specific pneumococcic serum was used in 156 (20.9%) of cases. In this group of 748 patients, 46 (6.2%)

* Statistics compiled by Martha Eaton, A.B.

died (Table 1). Forty-two of these deaths occurred among the 156 patients who were severely ill and were treated with combined serum and sulfadiazine therapy.

It is important to note from Table 1 that the patients in both series who received only sulfadiazine were of approximately the same frequency and same negligible mortality.

These results indicate that in the treatment of pneumococcic pneumonia with sulfadiazine, the adjuvant use of penicillin in place of specific serum in severely ill patients resulted in a decrease in mortality rate from 26.9% to 6.7%. The over-all mortality rate for pneumococcic pneumonia was decreased from 6.2 to 1.5%.

TABLE 1.—COMPARATIVE MORTALITY RATES, 1620 CASES OF PNEUMOCOCCIC PNEUMONIA, WITH SULFADIAZINE AND PENICILLIN OR SERUM AS ADJUVANT THERAPY

Therapy	No. cases	% incidence	No. deaths	% mortality
Sulfadiazine only	677	77.6	0	0.0
Penicillin and sulfadiazine	195	22.4	13	6.7
Total	872	100.0	13	1.5
Sulfadiazine only	592	79.1	4	0.7
Serum and sulfadiazine	156	20.9	42	26.9
Total	748	100.0	46	6.2

Comparative Severity of the Two Series. Since gross mortality statistics have little comparative value unless the series of cases represents illnesses of approximately equal severity, these two groups were analyzed as to the severity of the various important factors which influence the mortality in pneumococcic pneumonia.

It has already been shown (Table 1) that the two series were of similar clinical severity, since about 78% of each group was judged of insufficient severity to require adjuvant therapy, and the fatality rate was comparably negligible in these groups treated with sulfadiazine only.

Comparative Incidence and Mortality Rates of Specific Pneumococcic Types. It is apparent from Table 2 that the same specific pneumococcic types predominated in both groups; furthermore, in each type a marked decrease in the per cent mortality occurred with the addition of penicillin therapy. It is important to note that penicillin is extremely effective in the treatment of pneumococcic Type III pneumonia.

Comparative Age of Patients. As is demonstrated in Table 3, the age of a patient is a very important factor influencing mortality in pneumonia. Table 3 further shows that the comparative incidence according to age agrees very closely in the two series. It is of interest that 51% of the patients in both series were over forty years of age. In the penicillin treated series the per cent mortality was lower in every decade.

Comparative Severity of Associated Diseases. Obviously the presence of an associated disease may be very important in influencing the mortality of pneumonia. The grading of the severity of an associated disease is difficult and depends upon the judgment of the examiner. Some uniformity of grading was attempted by grading an associated disease as "mild" if it was sufficiently severe to aggravate or alter the course of the pneumonia, "moderate" if it was sufficiently severe so that associated with pneumonia it might jeopardize the life of the patient, and "severe" if the associated disease was in itself sufficient to endanger the life of the patient. Table 4 shows that associated diseases were present with about the same frequency in both groups. Although associated diseases graded as "severe" were less frequent in the penicillin treated series, it is apparent that the marked decrease in mortality in this group is

primarily due to adjuvant penicillin therapy.

Tables 1 through 4 indicate that these two series of cases represent groups of approximately equal severity, and therefore their mortality statistics can be directly compared.

Comparative Incidence of Complicating Conditions. As shown in Table 5 the incidence of important complicating conditions was equally low in the two series. Except for an increased frequency of sterile effusions, the additional use of penicillin did not materially alter the incidence of common complicating conditions.

Pleural empyema developed in 1 patient in the penicillin treated group. Injections of penicillin into the empyema cavity failed to cure the infection and a rib resection with drainage was performed resulting in prompt recovery.

Serofibrinous pericardial effusions developed in 3 patients as evidenced by the recognition of a pericardial friction rub,

TABLE 2.—COMPARATIVE INCIDENCE AND MORTALITY OF COMMON SPECIFIC PNEUMOCOCCIC TYPES

Pneumo. type	No. cases	% incidence	No. deaths	% mortality
A. Combined Penicillin and Sulfadiazine Therapy (195 Cases)				
VII	33	16.9	1	3.0
III	27	13.8	2	7.4
I	22	11.3	1	4.5
IV	15	7.7	2	13.3
VIII	13	6.7	0	0.0
XII	10	5.1	1	10.0
II	9	4.6	1	
V	8	4.1	1	
XXV	5	2.6	1	
XXIV	3	1.5	0	
VI	2	1.0	0	
X	2	1.0	0	
XIV	2	1.0	1	
XVII	2	1.0	0	
XXXIII	2	1.0	0	
B. Combined Serum and Sulfadiazine Therapy (156 Cases)				
VII	50	32.2	9	18.0
III	30	19.3	15	50.0
I	17	10.9	4	23.5
V	10	6.4	2	20.0
VIII	10	6.4	0	0.0
XXV	10	6.4	0	0.0
XII	7	4.5	2	
II	6	3.8	2	
IV	6	3.8	4	
XX	4	2.6	0	
XXIV	2	1.3	1	
VI	1	0.6	0	
XIV	1	0.6	0	
XIX	1	0.6	1	
C. Sulfadiazine With Adjuvant Penicillin Therapy (872 Cases)				
VII	81	9.3	1	1.3
I	71	8.1	1	1.4
III	46	5.3	2	4.3
XXV	43	4.9	1	2.4
IV	41	4.7	2	4.2
VIII	41	4.7	0	0.0
V	35	4.0	1	2.9
XII	35	4.0	1	2.9
II	29	3.3	1	3.4
XXIV	27	3.1	0	
XX	20	2.3	0	
IX	13	1.5	0	
VI	12	1.4	0	
XVIII	12	1.4	0	
XIII	11	1.3	0	
D. Sulfadiazine With Adjuvant Serum Therapy (748 Cases)				
VII	92	12.3	10	10.9
XXV	55	7.4	1	1.8
I	52	7.0	6	11.5
III	47	6.3	16	34.0
V	45	6.0	2	4.4
VIII	32	4.3	0	0.0
IV	31	4.1	4	12.9
XII	30	4.0	2	6.7
XXIV	13	1.7	1	7.7
XXXII	13	1.7	0	
XX	11	1.5	0	
XXXIII	11	1.5	0	
II	10	1.3	2	
XVIII	10	1.3	0	
XIX	10	1.3	1	

TABLE 3.—COMPARATIVE INCIDENCE AND MORTALITY BY AGE

Age (yrs.)	No. cases	% incidence	No. deaths	% mortality
A. Combined Penicillin and Sulfadiazine Therapy				
Under 20	7	3.6	0	0.0
20-29	27	13.8	0	0.0
30-39	33	16.9	1	3.0
40-49	53	27.2	4	7.7
50-59	43	22.1	6	14.0
60-69	25	12.8	1	4.0
70 and over	7	3.6	1	14.3
Total	195	100.0	13	6.7
B. Combined Serum and Sulfadiazine Therapy				
Under 20	6	3.8	0	0.0
20-29	22	14.1	3	13.6
30-39	30	19.2	4	13.3
40-49	34	21.9	10	29.4
50-59	45	28.9	17	35.6
60-69	18	11.5	7	38.9
70 and over	1	0.6	1	100.0
Total	156	100.0	42	26.9
C. Sulfadiazine With Adjuvant Penicillin Therapy				
Under 20	45	5.2	0	0.0
20-29	157	18.0	0	0.0
30-39	221	25.4	1	0.5
40-49	209	24.0	4	1.9
50-59	145	16.6	6	4.1
60-69	85	9.7	1	1.2
70 and over	10	1.1	1	10.0
Total	872	100.0	13	1.5
D. Sulfadiazine With Adjuvant Serum Therapy				
Under 20	49	6.6	0	0.0
20-29	141	18.8	3	2.1
30-39	177	23.7	5	2.8
40-49	172	22.9	10	5.8
50-59	126	16.9	18	14.3
60-69	75	10.0	9	12.0
70 and over	8	1.1	1	12.5
Total	748	100.0	46	6.2

roentgenographic demonstration of a transitory diffuse enlargement of the heart, and characteristic changes in the electrocardiogram. In each of these patients the pericardial effusion absorbed spontaneously. No instances of meningitis developed in either series.

Comparative Length of Hospital Stay. Excluding fatal cases, about two-thirds of the patients in each group had recovered from their acute illness and were discharged from the hospital in less than 7 days (Table 6). Only about 8% of patients in each group required over 14 days of hospital care. No remarkable difference in length of hospital stay was noted for severely ill patients treated with penicillin as compared with those treated with serum.

Indications for Penicillin Therapy. Although it is likely that in the future, penicillin will be administered routinely to all patients with pneumococcic pneumonia, the restricted supply of penicillin during the past year has limited the use of the drug to severely ill patients only.

In this series of cases, indications for *combined* penicillin and sulfadiazine therapy were:

1. Patients with high pneumococci counts in their sputum, with evidence of impending pulmonary edema or shock, with severe toxic delirium, with a leukocyte count below 6000 cells per cmm;[7] or other evidence of severe infection or toxicity.

2. Failure to respond to adequate dosage of sulfadiazine (studies[8,9] have demonstrated sulfadiazine-fast pneumococci to be readily susceptible to penicillin).

3. Pneumococcic Type III pneumonia in patients over 40 years of age.

In the above groups, not only were maximum doses of penicillin given in order to obtain immediate antibacterial action,[4] but high blood concentrations of sulfadiazine were also maintained, since it has been shown that combined sulfonamide and penicillin therapy is more effective than either sulfadiazine or penicillin alone.[7,14]

Penicillin therapy *alone* was given to patients with:

1. A previous history of sulfonamide sensitivity or who developed sensitivity to sulfadiazine before the pneumonic process had cleared.

2. Cardiac failure, renal insufficiency, or acidosis.

Penicillin Administration. In the majority of patients who received penicillin, the drug was administered intramus-

TABLE 4.—COMPARATIVE SEVERITY OF ASSOCIATED DISEASES

Associated diseases (grade severity)	No. cases	% incidence	No. deaths	% mortality	No. cases	% incidence	No. deaths	% mortality
	Sulfadiazine With Adjuvant Penicillin Therapy				*Sulfadiazine With Adjuvant Serum Therapy*			
Present	193	22.1	9	4.7	142	19.0	15	10.6
Mild	100	51.8	1	1.0	34	36.3	2	5.9
Moderate	77	39.9	5	6.5	43	46.2	0	0.0
Severe	16	8.3	3	18.8	16	17.2	12	75.0
Total (graded)	193	100.0	9	4.7	93	100.0	14	15.1
Severity not graded	0	..	..	..	49	..	1	2.0
Not present	679	77.9	4	0.6	606	81.0	31	5.1
Total	872	100.0	13	1.5	748	100.0	46	6.2

TABLE 5.—COMPARATIVE INCIDENCE OF COMPLICATING CONDITIONS

Complication	Sulfadiazine with adjuvant penicillin therapy		Sulfadiazine with adjuvant serum therapy	
	No. cases	% incidence	No. cases	% incidence
Sterile effusion	47	5.4	20	2.7
Empyema	1	0.1	2	0.3
Lung abscess	2	0.2	4	0.6
Pericarditis	3	0.3	2	0.3
Endocarditis	0	0.0	2	0.3

cularly in doses of 25,000 units every 3 hours. To prolong the effect of penicillin by delaying the rate of absorption (and to decrease the pain of repeated injections), ice bags were applied to the site before and after the injection.[13] The penicillin was administered in a solution of physiologic saline or of 5% glucose in water.[1] Frequently the penicillin was given in doses of 100,000 to 200,000 units daily by continuous intravenous drip. The dosage and mode of administration varied with the severity of the illness; critically ill patients were given higher doses more frequently. Continuous intravenous drip appeared to be the most efficient method of administration.[10]

The majority of patients received a total of about 500,000 units of penicillin. A small group of patients who died shortly after admission received less than 100,000 units because of the short period of treatment (Table 7).

Penicillin therapy was usually continued until the patient was afebrile at least 48 hours. To avoid relapses it was found necessary to continue penicillin administration for a somewhat longer

TABLE 6.—COMPARATIVE LENGTH OF HOSPITAL STAY (EXCLUSIVE OF DEATHS)

Hospital days	No. cases	% incidence	No. cases	% incidence
	A. Combined Penicillin and Sulfadiazine Therapy		*B. Combined Serum and Sulfadiazine Therapy*	
Under 7	49	26.9	24	21.1
7–14	80	44.0	53	46.5
15–21	20	11.0	20	17.5
22–28	11	6.0	5	4.4
Over 28	22	12.1	12	10.5
Total	182	100.0	114	100.0
	C. Sulfadiazine With Adjuvant Penicillin Therapy		*D. Sulfadiazine With Adjuvant Serum Therapy*	
Under 7	582	67.8	461	65.8
7–14	204	23.7	184	26.1
15–21	32	3.7	29	4.1
22–28	18	2.1	11	1.6
Over 28	23	2.7	17	2.4
Total	859	100.0	702	100.0

TABLE 7.—PENICILLIN THERAPY

	No. cases	% incidence	No. deaths	% mortality
A. Method of administration:				
Intravenous	30	15.4	3	10.0
Intramuscular	90	46.2	5	5.6
Intravenous and intramuscular	75	38.4	5	6.7
Total	195	100.0	13	6.7
B. Total dosage (units):				
Less than 100,000	10	5.1	2	20.0
100,000–499,000	100	51.3	7	7.0
500,000–1,000,000	55	28.2	1	1.8
More than 1,000,000	30	15.4	3	10.3
Total	195	100.0	13	6.7
C. Toxic reaction:				
Local	7	3.6	0	0.0
General: Urticaria	2	1.0	0	0.0
Dermatitis	1	0.5	0	0.0
Fever	1	0.5	0	0.0
No reaction	184	94.4	13	7.1
Total	195	100.0	13	6.7

period than was ordinarily considered as sufficient for sulfadiazine. This may be because penicillin is excreted within a few hours, whereas therapeutic doses of sulfadiazine are not entirely excreted within 24 hours. Tillett[12] also advised continuing penicillin therapy for at least 3 to 4 days of illness so as to avoid relapses due to discontinuing the drug before the development of type specific immunity.

Toxic reactions to penicillin were rare, but about 10% of patients receiving intravenous penicillin developed local thrombophlebitis. Two patients developed generalized urticaria which promptly cleared after cessation of penicillin therapy. One patient developed a maculopapular and vesicular itching eruption on the 9th day of therapy which spread from the trunk to thighs in spite of discontinuing sulfadiazine and all medications except penicillin; the eruption cleared on the 15th day, 2 days after stopping penicillin therapy. One patient developed a febrile reaction; after this patient had a normal temperature for for 3 days, 60,000 units of penicillin as a test dose were administered intramuscularly over a 12-hour period and a transitory rise in temperature to 100.5° F. resulted.

Causes of Failure in Treatment. Of the 13 patients in this series who died, 5 were admitted to the hospital *in extremis* with signs of pulmonary edema and severe shock, and died 4, 8, 9, 15 and 17 hours following admission. The 1st of these patients had associated rheumatic heart disease with early congestive failure and chronic glomerulonephritis (confirmed by autopsy). The 2d was a severe chronic alcoholic. The 3d was also a severe chronic alcoholic, with advanced hepatic cirrhosis (confirmed by autopsy).

The 6th fatal case, a patient aged 43, with a pneumococcic Type XXV pneumonia involving the entire right lung had associated hypertensive heart disease (confirmed at autopsy) with a 6 months history of paroxysmal nocturnal dyspnea. Although he was treated with high doses of sulfadiazine (attaining a blood concentration of 26.3 mg. per 100 cc.) and 200,000 units of penicillin intravenously daily, he failed to respond and died 36 hours after admission.

The 7th fatal case was that of a patient aged 58 with extensive right upper lobe pneumonia. Sputum examination showed the presence of much mixed infection, but many pneumococci were present which did not react to sera Types I to XXXIII. The patient was a confirmed chronic alcoholic, and was obviously jaundiced on admission, with a large, tender, palpable liver and an icterus index of 19.7 units. He was treated with sulfadiazine in high doses (attaining a blood concentration of 24 mg. per 100 cc.) and penicillin up to 300,000 units daily. He showed no evidence of response to therapy, rapidly going into pulmonary edema and shock and died 43 hours after admission. Autopsy revealed the presence of a markedly fatty liver.

The 8th patient, aged 62, was admitted with pneumococcic Type IV pneumonia involving the left lower lobe and the entire right lung. He was treated with high doses of sulfadiazine and penicillin, but died 30 hours after admission. Autopsy examination revealed the presence of carcinoma of the stomach, postoperative, with recurrence and metastases to liver, pancreas, and retroperitoneal lymph nodes.

The 9th fatality, a 59 year old man with chronic lymphatic leukemia with leukemic pulmonary infiltrations (confirmed at autopsy) was admitted with pneumococcic Type III lobar pneumonia. He was treated with large doses of sulfadiazine (blood level 22.4 mg. per 100 cc.) and penicillin (200,000 units daily) without response, and died in pulmonary edema 5 days after admission.

Although the 6th, 7th, 8th and 9th fatal cases might have benefited by massive doses of penicillin, the fulminating nature of the pneumonia and the severity of the associated diseases made recovery in each case unlikely. This problem has been presented by Bloomfield *et al.*[2]

The 10th patient, aged 74, with pneumococcic Type I bacteremia and pneumonia involving the left lower lobe, received sulfadiazine on the 1st day of treatment only because of associated diseases. Although

the patient received 25,000 units of penicillin intramuscularly every 3 hours during his entire hospital stay, he failed to respond to therapy and died on the 6th day.

The 11th fatal case, a patient aged 59, with bilateral pneumococcic pneumonia, was treated with moderate doses of sulfadiazine for 3 days, during which time a sulfadiazine blood level was 6.6 mg. per 100 cc. On the 4th day high doses of sulfadiazine were administered, and on the 5th day he was started on continuous intravenous drip of penicillin, receiving 200,000 units daily. However, the pneumonia spread rapidly and the patient died with pulmonary edema on the 6th day.

The 10th patient, above, did not receive the benefit of combined sulfadiazine and penicillin therapy. The 11th patient was treated with too low levels of sulfadiazine the first few days; at present with the more generous amounts of penicillin available, penicillin also would probably have been started much earlier.

The 12th patient, aged 54, with pneumococcus Type III pneumonia involving the entire right lung, also had a leukopenia of 1600 white cells per cmm. High doses of sulfadiazine were administered (reaching blood concentrations to 33 mg. per 100 cc.) and penicillin intermittently in doses of 25,000 units intramuscularly. After several days of therapy, the patient was unimproved, and the sputum was still loaded with Type III pneumococci. Sulfathiazole was then administered because the organism was obviously sulfadiazine-resistant, but no response was noted. The patient died on the 14th hospital day in pulmonary edema, with a complicating sterile right pleural effusion.

It is evident that the organisms in this patient were sulfonamide-fast, and much larger doses of penicillin by continuous intravenous drip might have been of value.

The 13th fatal case was a patient 40 years of age, with bilateral pneumococcic Type II pneumonia who was treated with high doses of sulfadiazine (blood concentration 16.4 mg. per 100 cc.) for 7 days. Sulfathiazole was begun on the 8th day because of his failure to improve, and discontinued on the 14th day because of the development of a severe sulfathiazole rash. Penicillin had been administered by continuous intravenous drip, averaging 150,000 units daily beginning on the 5th hospital day. In addition, because the organisms were obviously sulfonamide and penicillin resistant, on the 15th day 200,000 units of pneumococcic Type II serum were administered. The patient also had an associated acute glomerulonephritis (confirmed at autopsy) with progressive uremia, and developed an acute pericarditis with effusion. The patient died on the 20th day in a state of uremia.

This patient might have benefited by larger doses of penicillin; however, at the autopsy examination it was obvious that the associated glomerulonephritis in itself might have been fatal.

A review of these fatal cases indicated that causes of failure in combined sulfadiazine and penicillin therapy were due to:

1. Patients who were admitted to the hospital *in extremis* and died within a few hours.

2. Inadequate doses of either sulfadiazine or penicillin, or both, due to:

   (*a*) Inadvisability of administration because of drug sensitivity;

   (*b*) Insufficient amount of drug given, or

   (*c*) Development of resistance of the organism to the blood concentrations of drug present.

3. The presence of severe associated diseases which in themselves could be fatal.

Summary and Conclusions. An analysis of 1620 consecutive patients with pneumococcic pneumonia indicated that combined penicillin and sulfadiazine therapy was most effective, with a resultant gross mortality rate of 1.5%.

Penicillin was very effective in the treatment of Type III pneumococcic pneumonia.

The use of penicillin did not decrease the incidence of complicating conditions nor shorten the length of hospital stay.

Details of penicillin therapy were outlined, including its indications, modes of administration, dosage, toxic reactions, and causes for failure of treatment.

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THE INFLUENCE OF SULFANILAMIDE THERAPY UPON THE COURSE OF ACUTE GLOMERULONEPHRITIS IN CHILDREN

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Ideally the treatment of acute glomerulonephritis during the initial stage of the disease should control the agent responsible for the initiation of the disease and correct the biochemical and physiologic derangements brought about by the disease. This report deals solely with the results obtained in the attempt to influence the process which is almost universally believed to be related causally to the disease, *viz.*, focal infection. The bacteriologic and immunologic studies of Longcope[2] and his coworkers have firmly established the opinion which Löhlein[1] stated in 1907, that streptococcal infection precedes with great frequency the onset of acute glomerulonephritis. Because of the finding of infection with the beta hemolytic streptococcus in the majority of cases of acute glomerulonephritis, it is now widely held that extrarenal focal infection with this organism is usually responsible for the production of the disease in the kidneys.

This study, begun in 1938, reports the influence of therapy with sulfanilamide, a drug highly effective against the beta hemolytic streptococcus, upon the course of 33 patients with acute glomerulonephritis. When less toxic sulfonamide compounds became available, they were not substituted for sulfanilamide because of the desire to avoid the complicating crystalluria and hematuria attending the use of these compounds, a complication which is rarely if ever caused by sulfanilamide. In addition, the low renal toxicity of sulfanilamide demonstrated by Marshall and Emerson[4] would recommend this drug as a safe chemotherapeutic agent in a patient with renal disease. Forty patients with acute glomerulonephritis to whom sulfanilamide was not administered but who were treated similarly in all other respects served as a control group. The control group of 40 patients formed the basis for a previous report on the criteria for clinical recovery in acute glomerulonephritis in children.[6] In assessing recovery from acute glomerulonephritis in children we have employed measures which we believe best indicate the status of both the anatomic inflammatory process in the kidneys and the functional disturbances attending the renal lesion. The persistence of the anatomic inflammatory changes in the kidney was gauged by routine urinalyses and Addis urinary sediment counts done frequently. In reporting the results of the Addis sediment count, we have used the values for red blood cell excretion, since we have found these to be the last of the formed urinary "elements" to return to normal. In addition the red blood cell sedimentation rate has been followed in these patients. It is not possible to designate with certainty the factors responsible for the prolongation of the abnormal sedimentation rate in patients with acute glomerulonephritis. The rapid sedimentation rate closely parallels the presence of an abnormal urinary sediment and may conceivably be dependent upon the presence of active inflammatory change in the kidneys. However, the possibility that the continuation of the original focal infection in a subclinical degree not readily apparent, may account for the persistence

of the rapid red blood cell sedimentation rate cannot be excluded. The major functional disturbances resulting from the renal disease are derangements of renal function and functional abnormalities in the cardiovascular system chiefly dependent upon vascular spasm. The functional status of the kidney was measured by both the urea clearance test and the phenolsulfonphthalein excretion test. The return of the blood pressure to a normal level was taken as an indication of the disappearance of vascular spasm. Frequent clinical, roentgenographic, and electrocardiographic examinations of the heart served as a guide in determining the status of this organ.

8 days in the sulfanilamide treated group and 6 days in the untreated group. The proportion of patients with clinically apparent cardiac involvement was almost the same in both groups, 10 patients or 30% in the sulfanilamide treated group, and 13 patients or 32% in the control group. Similarly there were 20 patients or 60%, with significant hypertension in the sulfanilamide treated group and 23 patients or 57% in the controls. While there is no precise method for expressing quantitatively, the severity of the disease, when the patient is first seen, the overall impression of both groups with respect to the degrees of severity of nephritis on admission to the hospital was that they

TABLE 1.—DATA ON THE TREATED AND UNTREATED GROUPS

	Untreated group	Sulfanilamide treated group
Number in groups	40	33
Average age	6 ± 3 yrs.	6 ± 3.1 yrs.
Duration of nephritis before admission to hospital	6 days (1 to 29 days)	8 days (1 to 35 days)
Cardiac involvement (clinically evident)	13	10
Hypertension	23	20

TABLE 2.—ORGANISMS ISOLATED IN THE TWO GROUPS OF PATIENTS

Predominant organism	Untreated group (40)	Sulfanilamide treated group (33)
Streptococcus hemolyticus	24	18
Staphylococcus aureus	10	7
Pneumococcus	2	4
Streptococcus viridans	4	3
Micrococcus catarrhalis	0	1

The two groups of children with acute glomerulonephritis were similar in so many respects that they are comparable samples. About half the children in each group were negroes which is in keeping with the proportion of colored children admitted to the Children's Hospital of Philadelphia for all causes. The average age of both groups was approximately 6 years. In both groups there was a slight preponderance of males —57%. Osman[5] showed that the preponderance of males affected by this disease is significant in every age group except that from 16 to 20 years. The duration of the disease before admission to the hospital, and thus initiation of treatment, was not appreciably different, averaging

did not differ appreciably. Table 1 summarizes some of the characteristics of both groups of children at the time of admission to the hospital. Both groups of patients were hospitalized continuously until they were judged to be free from their disease. The treatment of all patients other than the administration of sulfanilamide was similar.

Routine nasopharyngeal and blood cultures were taken on all patients at the time of admission to the hospital. In addition cultures of any infected skin lesions or purulent discharges were obtained. Table 2 presents the results of the nasopharyngeal cultures. While many of the patients had a variety of organisms in

their upper respiratory tracts, only the predominant organism is recorded. The beta hemolytic streptococcus was the organism most frequently encountered, 60% of the untreated group and 54% of the sulfanilamide treated group. Unfortunately, repeated cultures were not done, for it is likely that a higher incidence of streptococcal infection would have been revealed in this way. In only 1 instance a patient, who had an antecedent history of scarlet fever was a positive blood culture obtained (beta hemolytic streptococcus). It may be assumed that infection with the beta hemolytic streptococcus was present in many patients from whom the organism was not isolated in culture, since Longcope[3] has demonstrated with great regularity a marked rise in the antistreptolysin titer of the blood during the course of acute glomerulonephritis, even when cultures failed to show hemolytic streptococci.

Sulfanilamide was administered orally according to a fixed plan. Every patient received 1 gr. per pound of body weight daily for 5 days, following an initial dose of half the calculated total daily dose. The drug was then administered at half this dosage level for an additional 15 days, and finally a maintenance dose of 10 to 20 gr. daily was given for variable periods of 2 to 6 weeks. Thus sulfanilamide was given over a period of 34 to 62 days. Blood sulfanilamide concentrations measured in 19 patients varied from 4 to 16 mg. per 100 cc.—in only 2 patients did the blood concentration exceed 10 mg. per 100 cc. Hemoglobin determinations and white blood cell counts were made every third day for 12 days and at weekly intervals thereafter.

Most of the patients were cyanotic during the first week of therapy. Two patients developed a marked drop in hemoglobin which was treated by blood transfusions without stopping the sulfanilamide. One child developed on the third day of drug administration a weeping cutaneous eruption with involvement of the mouth, conjunctivæ, vagina and perineal region (a type of erythema multiforme which has been termed ectodermosis erosiva pluri-

TABLE 3.—RATES OF RETURN TO NORMAL OF FINDINGS INDICATIVE OF PERSISTENCE OF RENAL LESION

	Untreated group (days)	Sulfanilamide treated group (days)
Routine urinalysis	37 ± 18	32 ± 17
Addis count:		
10,000,000 red cells	76 ± 32	63 ± 29
Less than 1,000,000 red cells	120 ± 75	86 ± 69
Sedimentation rate	86 ± 48	71 ± 46

TABLE 4.—RATES OF RETURN TO NORMAL OF ALTERED PHYSIOLOGIC FUNCTIONS

	Untreated group (days)	Sulfanilamide treated group (days)
Renal function	19	21
Heart	14	10
Blood pressure	7	8.5

orificialis). No severe leukopenias were encountered.

Other than local therapy for cutaneous infection (impetigo) and myringotomy when indicated, no other attempt at removal of foci of infection was made during the study of both groups of children.

Results. The value of sulfanilamide administration in the treatment of the patient with acute glomerulonephritis may be examined from two aspects, first, its effect on the infectious episode which precedes and presumably precipitates the renal disturbance and, second, the influence upon the course and duration of the renal disease. While physical examinations showed that the original infections were controlled and clinically arrested by

the drug, since serial nasopharyngeal cultures were not done we cannot state when the bacterial infection was terminated. The data accumulated in this study pertain solely to the influence of sulfanilamide upon various manifestations of the diseased kidneys and the duration of the inflammatory process in the kidneys. In Table 3, there are presented the average durations of the abnormalities in routine urinalysis, Addis urinary sediment count, and sedimentation rate for both the sulfanilamide treated and control groups.

The routine urinalysis became normal in both groups in about the same length of time—37 days in the untreated group, and 32 days in the sulfanilamide treated group. The Addis urinary sediment count, reporting excretion of red cells only, seemed to return to normal somewhat sooner in the sulfanilamide treated group, an average of 86 days as opposed to 120 days in the control. However, because of the large values for the standard deviation, this difference is not statistically significant. The red blood cell sedimentation rate returned to normal in 71 days in the sulfanilamide treated group and in 86 days in the untreated group. Again, because of the wide variability in each group, the difference in these two average values has no statistical significance. It seems reasonable to conclude that sulfanilamide administration was without influence on the duration of the inflammatory lesion in the kidney. Complete recovery from acute glomerulonephritis occurred in all patients in both groups.

Serial estimations of renal function by the urea clearance test or phenolsulphonphthalein excretion test or both were made in the 2 groups of patients. Sulfanilamide administration had no influence upon the time required for the restoration of normal kidney function, as measured by these tests, 21 days in the treated group and 19 days in the untreated group (Table 4). This finding may also be regarded as additional evidence that sulfanilamide in therapeutic doses does not depress renal function in these patients.

Since hypertension, which occurred in 60% of all the patients is an important etiologic factor in the serious complications, heart failure and hypertensive encephalopathy, it is of interest to note that the duration of hypertension was not influenced by sulfanilamide administration. The blood pressure attained normal levels in 7 days in the untreated group and 8.5 days in the treated group (Table 4).

Of the entire group of patients, 31% presented evidences of cardiac abnormalities which were apparent on physical examination. The return to a normal cardiac status in these patients was determined by repeated physical, roentgenographic, and electrocardiographic examinations. There was no significant difference in the duration of cardiac abnormality in the 2 groups (Table 4). Thus sulfanilamide therapy did not affect the course of the above mentioned physiologic derangements which so frequently accompany acute glomerulonephritis.

Since acute glomerulonephritis in children in marked contrast to this disease in adults rarely progresses to a chronic stage, it is obvious that the duration of the illness rather than the incidence of chronicity must serve as a criterion in assessing the action of sulfanilamide therapy in childhood. This difference in the natural history of the disease between the child and the adult, may account for the differences in therapeutic results obtained in this study and those reported by Williams *et al.*[7] The reasons for this difference in the course of acute glomerulonephritis in the child and adult are not apparent and are certainly worthy of further study.

The production of the renal lesion of acute glomerulonephritis generally is believed to result from an allergic reaction in the kidney dependent upon an extrarenal focus of infection. Whether the severity and persistence of the antecedent infection bear any relation to the severity and persistence of the acute renal lesion is not clear. If there were a high degree of correlation between these two processes, then the administration of sulfanilamide

would be expected to decrease the severity and duration of the acute renal episode because of the suppression of the focus of infection. Since sulfanilamide administration did not decrease the severity or duration of the disease in the children studied, it is possible that factors other than original focus of infection may be operative.

Summary and Conclusions. 1. A group of 33 children with acute glomerulonephritis, treated with sulfanilamide was compared to a group of 40 children with the same disease, not given this drug. Both groups were comparable in age, sex, duration of illness prior to hospitalization, severity of renal disturbance, in the incidence of accompanying cardiovascular derangements, and in the predominant infecting organism (the beta hemolytic streptococcus).

2. All children in both groups recovered completely from their renal disease.

3. There was no significant difference in the duration of the renal inflammatory process as estimated by the routine urinalysis, Addis urinary sediment count, and red blood cell sedimentation rate.

4. There was no statistically significant differences between the duration of the disturbed renal or cardiovascular functions in the two groups.

5. Sulfanilamide had no demonstrable deleterious effect on the renal function and did not cause an unusual incidence of toxic phenomena in patients with acute glomerulonephritis.

6. Sulfanilamide therapy appears to be without influence on the course and duration of acute glomerulonephritis in childhood. This does not deny the value of sulfanilamide (and other sulfonamides) in the treatment of infection in the patient with nephritis.

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THE TREATMENT OF TEMPORARY RENAL INSUFFICIENCY (UREMIA) BY PERITONEAL LAVAGE

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Even though the syndrome known as uremia occurs fairly frequently and the diagnosis can be made quite readily there is no positive proof as to the cause of death in this condition. As Harrison and Mason[13] have pointed out, numerous theories have been advanced, and it seems plausible that various factors might lead to death in different patients, or that a combination of factors may be present. Death therefore seems to result from an accumulation of toxic products, changes in the osmotic pressure of the body fluid, dehydration or overhydration, an acid-base imbalance or a combination of one or more of the aforementioned causes.[9,13]

The primary function of the kidney is to eliminate waste or toxic products (glomerular function) and at the same time maintain a normal electrolyte and water balance (largely dependent on tubular function). Regardless of the cause of uremia it is therefore evident that in treating such a syndrome successfully a normal internal environment must be maintained and all the aforementioned items must be kept in mind. Attempts have been made to treat both experimental animals[5,11,12] and patients[19,25] by peritoneal lavage but the results to date have not been too satisfactory, apparently due to the fact that the water and electrolyte balance was not sufficiently considered. It has been emphasized[6] that such a method should be employed only in cases that have a temporary and severe renal dysfunction. Patients suffering from bichloride of mercury poisoning, or from a toxic nephrosis resulting from the administration of one of the sulfonamide compounds would be good candidates for such therapy providing other measures had failed or were thought to be of no avail. Other possible applications for peritoneal lavage might well be in patients who were anuric following a hemolytic transfusion reaction or a crushing injury. Bywaters[7] has recently reviewed the literature on the "Crush Syndrome" and states that if anuria occurs the prognosis is grave. Therefore it seems possible that if such patients could be maintained for several days it might provide the necessary time for satisfactory kidney function to be restored. Moon[16,17] has felt that toxic products accumulate in certain types of shock, and if such is the case, it naturally follows that these toxic products pass through living membranes by dialysis. It therefore seems possible that they too could be eliminated from the body by peritoneal lavage.

In order to clarify the procedure to be used it was first necessary to settle the following points:

1. The method of carrying out peritoneal lavage: (*a*) continuous perfusion; or (*b*) intermittent injection and then withdrawal: (1) length of time fluid should remain in peritoneal cavity; (2) method of injection and frequency.

2. The selection of a proper solution with the following qualifications: (*a*) one which would permit a maximum diffusion of waste products into it; (*b*) one which would not alter the normal electrolyte pattern of the extracellular fluid; (*c*) one which would have the desired tonicity, and if possible; (*d*) a solution which would afford nutrition and thus keep the protein catabolism at a minimum.

With these problems in mind this study was undertaken in order to determine what solution could be best employed. It also seemed important to perfect the method of lavaging the peritoneal cavity so extremely large quantities of fluid would not have to be used and to use a solution which could be completely recovered.

Materials and Method. It was felt that intermittent washings of the peritoneal cavity would cause less difficulty and would be less apt to produce a state of overhydration. It was also thought advisable to use a sterile solution which chemically resembled an ultrafiltrate of plasma. In order to check these impressions it was necessary to establish at what time the maximum diffusion of nitrogenous products occurred and what solution could be best tolerated. Therefore peritoneal lavage employing a 5% solution of dextrose in distilled water, of Ringer's, Hartmann's, and "A" solutions were carried out. The composition of the latter 3 solutions is shown in Table 1.

Normal mongrel dogs were employed for this study and peritoneal lavage was accomplished by injecting the selected fluid through a large gauge needle (No. 15) which had been inserted through the abdominal wall after anesthetizing a small area with 1% procaine. One hundred cc. per kilogram of body weight of the lavage fluid was given intraperitoneally in the first 10 animals. Frequent blood and ascitic fluid samples were taken for analysis at the time interval following injection as shown in Tables 2, 3, 4 and 5.

Following the selection of the method and the solution to be employed ("A" solution) bilaterally nephrectomized dogs were used to further evaluate the procedure. All blood samples except the CO_2 determinations were obtained by employing greased syringes. Samples for the CO_2 content were obtained under oil, and stasis was avoided. Heparin was employed as the anticoagulant for all samples.

The hematocrit was determined by employing the Sanford and Magath cell volume tube.[21] In the normal animals the plasma protein concentration was calculated from the plasma specific gravity as advocated by Weech[26] employing the falling-drop* method described by Barbour and Hamilton.[5] In the nephrectomized animals the plasma protein concentration was determined by the micro-Kjeldahl technique and corrected for the elevated non-protein nitrogen. (It was found that the plasma protein concentration as determined chemically did not agree satisfactorily with that calculated from the specific gravity when the blood urea nitrogen was above 100 mg. per 100 cc.) Plasma and ascitic fluid chlorides were determined by employing the modification advocated by Wilson and Ball[27] of the method described by Van Slyke.[22] The method of Van Slyke and Cullen[23] was employed for the blood and ascitic fluid urea nitrogen and the CO_2 content was determined as described by Van Slyke and Neill.[24] In the later experiments 1 kidney was first removed and subsequently after control blood studies had been obtained the remaining nephrectomy was performed in 10 to 15 minutes using a light intratracheal ether anesthesia.

Results. The blood and ascitic fluid alterations noted when 5% dextrose in distilled water was employed for lavage are shown in Table 2. It can be observed that a marked hemoconcentration occurred and that the plasma protein concentration was greatly increased while the plasma chloride concentration fell to very low levels. These changes were due to a shift of electrolytes into the ascitic fluid and water into the intracellular compartment with a resulting dehydration of the extracellular fluid phase. Table 3 shows the changes which occurred when Ringer's solution was used. In these animals only slight alterations were noted in the hematocrit and plasma protein concentration although some dilution apparently occurred. (The plasma chloride level increased slightly while the CO_2 content was decreasing.) The analysis of the ascitic fluid in these dogs (Nos. 3, 4 and 5) showed that considerable amounts of bicarbonate was lost from the extracellular fluid, while chloride (and probably also some sodium)

* Barbour and Hamilton Falling-drop Apparatus kindly furnished by Eimer and Amend & Co., New York, N. Y.

passed from the injected fluid into the extracellular fluid compartment. Tables 4 and 5 show the chemical alterations that occurred when Hartmann's solution and "A" solution (without added dextrose) was employed. It can be seen that very little change in the plasma and ascitic fluid concentrations occurred except for the transfer of urea nitrogen into the lavage fluid. Table 6 shows a summary of these results in the first 10 animals and it is evident that "A" solution and Hartmann's solution would be the most desirable ones to use. Since there is a tendency towards acidosis in uremia "A" solution was employed in the subsequent experiments because of the greater amount of available bicarbonate.

The blood studies obtained from nontreated bilaterally nephrectomized dogs are shown in Table 7. The average survival time of these animals was slightly over 4 days. All of these dogs showed a high blood urea nitrogen concentration prior to death. Approximately 24 hours after removal of the second kidney in a similar group of animals, peritoneal lavage was instituted. Results in 3 such dogs are

TABLE 1.—SOLUTIONS EMPLOYED FOR PERITONEAL LAVAGE

Solutes in gm. per 1000 cc. of distilled water	Ringer's	Hartmann's	"A" solution
Sodium chloride (gm.)	9.00	6.00	6.10
Calcium chloride (gm.)	0.25	0.20	0.23
Potassium chloride (gm.)	0.30	0.40	0.35
Sodium phosphate (monobasic) (gm.)	..	..	0.07
Magnesium chloride (gm.)	..	..	0.05
Sodium bicarbonate (gm.)	..	..	2.20
Lactic acid (cc.)	..	2.40	
Dextrose (gm.)	..	..	10–20
Total gm. per 1000 cc.	9.55	..	19–29

TABLE 2.—RESULTS OBTAINED FROM 2 DOGS LAVAGED WITH A SOLUTION OF 5% DEXTROSE IN DISTILLED WATER

					Lavage fluid		
Dog No.	Time	Hematocrit	Plasma protein conc. (gm./100 cc.)	Plasma chloride (m.eq./l)	Urea nitrogen (mg./100 cc.)	Chloride (m.eq./l)	CO_2 (vol. %)
1	Control	50.0	6.72	113.8	0	0	0
	5 min. after injection	..	..	..	2.1	9.1	3.6
	4 hours	73.0	12.07	86.2	14.1	75.8	23.6
2	Control	49.2	6.12	110.0	0	0	
	2 hours	62.4	8.54	93.8	4.81	44.5	
	3 hours	66.1	9.05	93.6	6.41	53.2	
	4 hours	68.9	9.46	89.9	7.05	59.3	
	6¾ hours	78.9	10.44	83.1	9.20	68.1	

TABLE 3.—RESULTS OBTAINED FROM 3 DOGS LAVAGED WITH RINGER'S SOLUTION

		Blood and plasma				Lavage fluid		
Dog No.	Time	Hematocrit	Plasma protein conc. (gm./100 cc.)	Plasma chloride (m.eq./l)	Plasma CO_2 (vol %)	Urea nitrogen (mg./100 cc.)	Chloride (m.eq./l)	CO_2 (vol. %)
3	Control	38.5	6.36	109.9	48.2	0*	164.0*	0*
	2 hours	41.0	5.62	116.7	..	11.1	146.2	
	3 hours							
	4 hours	41.6	5.41	117.0	41.7	12.9	135.7	36.5
	5 hours							
	6 hours	44.1	5.41	116.2	..	12.4	130.2	38.2
4	Control	47.2	6.45	112.1	49.4	0*	166.0*	0*
	2 hours							
	3 hours	40.5	5.91	116.9	..	5.8	134.8	
	4 hours	40.0	5.87	119.1	44.1	5.7	133.1	40.6
	5 hours	39.2	5.74	115.2	..	4.5	130.0	
5	Control	49.0	5.58	109.4	44.0	0*	163.0*	0*
	2 hours							
	3 hours	42.2	5.14	112.0	..	9.0	141.2	23.4
	4 hours	41.8	5.17	113.3	41.8	9.3	136.2	29.4
	5 hours	41.3	5.11	118.5	40.2	8.8	131.5	34.6

* The level of the individual constituent in the lavage fluid previous to administration.

shown in Tables 8 and 9. In Dog 11 (Table 8) 1500 to 2500 cc. of "A" solution without dextrose was instilled into the peritoneal cavity two or four times a day and withdrawn 3 to 5 hours later. This was done on the 2d, 4th and 7th days. It can be seen that relatively large amounts of urea nitrogen were removed. In Dog 12 (Table 8) the intermittent intraperitoneal injection and removal of 5000 to 6000 cc.'s of fluid was done daily. These 2 dogs (Nos. 11 and 12) showed a marked generalized edema by the 5th day following bilateral nephrectomy which increased and appeared to be the cause of death after $8\frac{1}{4}$ and $7\frac{1}{2}$ days respectively. Since fairly large amounts of the injected fluid was not withdrawn it was quite obvious that there was an increase in the animals' body water.

TABLE 4.—RESULTS OBTAINED FROM 2 DOGS LAVAGED WITH HARTMANN'S SOLUTION

		Blood and plasma				Lavage fluid	
Dog No.	Time	Hematocrit	Plasma protein conc. (gm./100 cc.)	Plasma chloride (m.eq./l)	Plasma CO_2 (vol. %)	Urea nitrogen (mg./100 cc.)	Chloride (m.eq./l)
6	Control	44.2	6.07	112.0	45.2	0*	115.0*
	1 hour	40.5	5.87	111.5	..	4.7	120.0
	2 hours	41.6	5.97	111.5	43.0	6.6	122.3
	3 hours	39.4	5.74	112.4	42.6	7.6	123.2
7	Control	45.1	6.12	110.7	..	0*	110.0*
	1 hour						
	2 hours	44.2	6.09	113.5	..	7.3	121.5
	3 hours						
	4 hours	44.4	6.19	116.3	..	6.8	120.6
	6 hours	43.3	5.99	111.9	..	5.6	119.4

* The level of the individual constituent in the lavage fluid previous to administration.

TABLE 5.—RESULTS OBTAINED FROM 3 DOGS LAVAGED WITH "A" SOLUTION

		Blood and plasma				Lavage fluid		
Dog No.	Time	Hematocrit	Plasma protein conc. (gm./100 cc.)	Plasma chloride (m.eq./l)	Plasma CO_2 (vol. %)	Urea nitrogen (mg./100 cc.)	Chloride (m.eq./l)	CO_2 (vol. %)
8†	Control	42.8	5.89	108.5	47.3	0*	113.0*	29.8*
	1 hour	39.8	5.66	108.6	..	4.9	121.8	35.9
	2 hours	38.2	5.58	108.5	46.0	7.2	122.5	41.6
	3 hours	35.8	5.34	110.1	..	7.9	124.8	
9	Control	41.2	6.25	112.7	51.0	0*	114.5*	57.7*
	1 hour							
	2 hours	40.6	6.02	112.6	..	10.7	122.3	60.0
	3 hours	39.1	5.78	113.8	49.2	12.0	123.5	55.3
	4 hours	39.3	5.81	113.0	49.6	11.6	123.5	56.7
10	Control	51.9	6.13	107.2	49.9	0*	119.5	43.3*
	1 hour							
	2 hours	53.0	6.30	108.6	45.0	9.0	121.0	50.8
	3 hours	52.3	6.23	109.2	48.3	9.8	123.0	53.4
	4 hours	50.2	6.04	109.6	50.1	9.2	121.4	51.2

* The level of the individual constituent in the lavage fluid previous to administration.
† In Dog 8 only 1.1 gm. of sodium bicarbonate/1000 cc. was used in the lavage fluid, instead of 2.2 gm./1000 cc. as in the lavage fluid employed in Dogs 9 and 10.

TABLE 6.—AVERAGE CHANGE OF THE CONSTITUENTS MEASURED IN THE BLOOD, PLASMA AND LAVAGE FLUIDS AFTER 4 HOURS FOR EACH OF THE SOLUTIONS EMPLOYED

	Blood and plasma				Lavage fluid		
Solution employed	% change in hematocrit	% change in plasma protein conc.	% change in plasma chloride conc.	% change in plasma CO_2	Increase in urea nitrogen conc. (mg./100 cc.)	M.eq./l change in chloride conc.	Vol. % change in CO_2 content
5% dextrose in distilled water	+43.1	+67.1	−21.3	..	+10.6	+67.6	+23.6
Ringer's	− 7.4	−10.4	+ 5.5	−9.7	+ 9.3	−29.3	+35.5
Hartmann's*	− 7.4	− 2.2	+ 2.7	−4.9	+ 7.2	+ 8.8	
"A" solution†	− 4.0	− 4.3	+ 1.3	−1.2	+10.4	+ 4.7	+ 8.1

* These values represent the average change (% or conc.) at the end of 3 hours in 1 animal and 4 hours in the other.
† Dog 1, Table 5 omitted from this calculation because only half the intended amount of sodium bicarbonate was used in the solution.

The results with Dog 13 are shown in Table 9. The "A" solution was made slightly hypertonic by the addition of dextrose (the chemical make-up of the fluid was comparable to that shown in Table 1). By such a procedure it is possible to withdraw slightly more fluid than had been injected and thus it was feasible to utilize parenteral fluids to help control the acid-base balance and the state of nutrition. Nine days following bilateral nephrectomy the animal appeared perfectly normal (no visible edema was evident), and the blood studies were within the desired range. In the evening of the 9th day the dog died suddenly following a transfusion of citrated blood. The caloric and protein intake was maintained fairly well by the dextrose present in the peritoneal fluid and by the administration of

TABLE 7.—BLOOD STUDIES ON BILATERALLY NEPHRECTOMIZED UNTREATED ANIMALS*

Dog No.	Time (in days)	Hematocrit	Plasma protein conc. (gm./100 cc.)	Plasma chloride (m.eq./l.)	Plasma CO_2 (vol.%)	Blood urea nitrogen (mg./100 cc.)
1	Control	52.0	6.72	110.6	45.3	12.9
	2	45.0	6.47	94.6	41.4	143.7
	4	41.0	6.28	72.2	36.0	256.0
2	Control	45.1	6.12	110.7	49.4	9.0
	1	31.0	5.89	115.3	38.1	61.2
	2	29.2	5.60	107.1	32.7	104.1
	4	28.1	5.50	100.9	51.0	254.0
	5	29.7	5.23	99.4	43.4	299.6
3	Control	43.6	7.55	109.1	48.0	7.7
	2	39.8	..	97.0	46.9	80.8
	3	42.3	6.80	78.0	..	240.0
4	Control	..	..	..	53.8	9.3
	2	..	..	..	39.5	116.0
	4	..	..	..	28.1	190.0

* Average survival time of entire control group of non-treated animals, 4+ days.

TABLE 8.—BLOOD STUDIES ON BILATERALLY NEPHRECTOMIZED ANIMALS TREATED BY PERITONEAL LAVAGE*

Dog No.	Time in days after bilateral nephrectomy	Hematocrit	Plasma protein conc. (gm./100 cc.)	Plasma chloride (m.eq./l)	Plasma CO_2 content (vol. %)	Blood urea nitrogen (mg./100 cc.)
11	Control	51.0	6.24	108.4	46.0	10.0
	2 A.M.	43.0	5.58	106.9	43.3	138.6
	2 P.M.	46.0	6.70	..	42.6	70.1†
	4	39.0	5.84	106.3	41.6	111.8
	7	41.3	..	100.2	40.7	200.7
	8	45.4	5.62	128.2	38.5	101.1‡
12	Control§	38.5	6.36	109.9	45.2	12.2
	Control¶	43.4	6.25	108.7	54.0	9.1
	1 A.M.	39.6	..	107.3	..	30.7
	1 P.M.	..	..	..	..	26.1
	2	38.3	..	100.7	55.5	35.0
	4 A.M.	..	4.38	99.0	50.4	38.7
	4 P.M.	..	..	..	..	30.7
	5	31.6	..	100.2	50.0	35.4
	7	31.3	4.24	103.0	42.4	63.9

* All blood studies drawn in the A.M. except those so designated.
† 6055 cc. lavage fluid removed during the previous 12 hours containing 5.34 gm. of urea nitrogen.
‡ 7600 cc. lavage fluid removed in the previous 6 hours containing 10.1 gm. of urea nitrogen.
§ Control studies before nephrectomy.
¶ Blood studies following unilateral nephrectomy.

TABLE 9.—DAILY STUDIES ON DOG 13 AFTER BILATERAL NEPHRECTOMY AND TREATMENT BY PERITONEAL LAVAGE

Days of experiment	Control	2	3	4	5	6	7	8	9
Oral or intravenous intake in cc.	..	100	300	50	750	750	..	550	
Fluid injected intraperitoneally, cc.	..	6050	6500	5000	5000	10,000	7500	7800	7900
Fluid withdrawn from peritoneum in cc.	..	6300	7300	5000	4300	10,600	7925	7800	8200
Serum CO_2 content, vol. %	47.0	48.1	46.8	42.9	47.0	44.7	41.6	50.6	
Serum chloride, m.eq. per liter	109.2	118.8	104.2	95.5	95.5	98.0	96.3	100.1	101.2
Serum protein conc., gm. per 100 cc.	6.82	..	..	..	..	..	..	..	6.55
Hematocrit	44.3	43.7	47.0	52.0	57.7	50.0	46.0		
Blood urea nitrogen, mg. per 100 cc.	10.3	71.1	73.5	60.0	73.1	86.0	58.0	62.2	54.3

plasma, amino acids and dextrose solutions intravenously. No solid food or water was permitted orally, but the animal was given small amounts of milk on several occasions.

In none of the animals was there evidence of peritonitis. The bilaterally nephrectomized dogs were all autopsied and no abnormalities in the peritoneal cavity were noted.

Discussion. The factors which lead to death when kidney failure occurs can apparently be corrected or prevented by employing peritoneal lavage, but naturally this type of therapy would only be effective over short periods of time (2 to 14 days) and the ultimate success would then depend on the restoration of adequate kidney function.

Ganter[11] in 1923 first introduced peritoneal lavage for the treatment of uremia. Previously it had been shown by Putnam[18] that the organic solutes (urea, creatinine, etc.) and the body electrolytes would dialyze across the peritoneal membrane. With this fact in mind Ganter, employing a physiologic solution of sodium chloride, showed improvement in dogs manifesting the uremic state. In 1927 Heusser and Werder[14] employed Ringer's solution and in summary concluded that the best results should be obtained by using a lavage fluid which approached the normal electrolyte composition of plasma. In 1932 Bliss, Kastler and Nadler[6] reported their results and showed that as much as 9.9 gm. of non-protein nitrogen could be removed from uremic dogs in less than 24 hours. In their series, untreated bilaterally nephrectomized dogs survived approximately 3 days while in two similar animals in which the peritoneal cavity was lavaged, life was maintained for 13 and 16 days. They used a solution of balanced salts and allowed it to remain in the peritoneal cavity for 10 minutes before removal. The cause of death in some of the animals which they treated was apparently due to the accumulation of massive peripheral and pulmonary edema. In the lavage fluid after removal they identified creatinine, urea, phosphates, sulphates, urochrome and traces of protein. Haam and Fine[12] were able to reduce significantly the mortality rate by employing peritoneal lavage in rabbits which were made anuric by administering 30 mg. of bichloride of mercury per kilogram of body weight. Wear, Sisk and Trinkle[25] in 1938 employed this method of treatment, experimentally and clinically, using a poorly buffered Hartmann's solution with a pH of 4.2. Their results showed promise inasmuch as they also demonstrated that large quantities of non-protein nitrogen could be removed. They believed the procedure was without morbidity or mortality, and emphasized the fact that it would be successful only if acidosis could be prevented. Most of the investigators who have employed this procedure have used solutions which were not comparable to plasma in composition, and as a rule perfused the peritoneal cavity by inserting two trochars or rubber catheters. Haam and Fine[12] on the other hand inserted the fluid, which was kept at body temperature, intraperitoneally through a small rubber catheter and then after 10 to 30 minutes removed it by syphonage. It was noteworthy that the lavage fluid attained a relatively high urea nitrogen concentration although the average length of time between injection and withdrawal was less than 30 minutes.

The procedure has been employed in at least 13 human cases[6,14,19,20,25] with one reported survival.[25] In most instances a definite reduction in the blood urea nitrogen concentration was obtained. The failures were thought to occur because of the existence of permanently diseased kidneys in most of the cases, and occasionally it was thought to be due to inadequate or incorrect treatment.

Although the series of animals presented here is small, it is believed that certain definite knowledge was gained which would permit the wider use of this method.

That dialyzation of large quantities of urea nitrogen into the lavage fluid will occur has been demonstrated (up to 10.1 gm. of urea nitrogen was removed in 16 hours). Our studies and those of others[5,6,7]

have shown that the normal daily output of urea nitrogen can be approximated or exceeded by the use of peritoneal lavage. It is appreciated that the symptoms of uremia are not a result of the high concentration of urea nitrogen when kidney function is impaired. It has been shown[9,10,13] that toxic products are formed, and that there is also a retention of protein breakdown products.[13] If such be the case since such products are dialyzable[6,11,18] it seems logical to assume that uremic patients can be benefited by peritoneal lavage. In our experiments the urea nitrogen concentration was taken as a relative index of the rate and quantity of dialyzation of such products.

The maximum diffusion of urea nitrogen into the lavage fluid apparently occurs in from 2 to 4 hours. Haam and Fine[12] and Rhoads[19] demonstrated a more rapid rate of diffusion when the blood urea nitrogen was significantly elevated. It thus seems that it is more desirable to employ the intermittent injection and withdrawal ($\frac{1}{2}$ to 4 hours) technique rather than a continuous perfusion.

It has been previously demonstrated[8,15] that a 5% dextrose solution causes many undesirable alterations and hence would not be a suitable lavage fluid although it was employed in some of the experiments done by Rosenak and Simon.[20] Ringer's solution would not be satisfactory because of the lack of bicarbonate and its high chloride concentration. The repeated lavaging with such a solution would result in a considerable loss of bicarbonate from the blood.

With the use of any of the isotonic salt solutions a retention of sodium and hence water is apt to occur and result in extreme overhydration and pulmonary edema. This has also been observed by others;[6] therefore we feel that by the addition of small amounts of dextrose, the lavage fluid is made slightly hypertonic and complete recovery of the injected fluid can be obtained. In fact, it is possible to withdraw somewhat larger quantities, thus permitting the use of oral feedings or solutions intravenously which contain constituents needed to maintain adequate nutrition (vitamins, amino acids, dextrose and electrolytes). It would seem quite desirable to provide an adequate carbohydrate intake so that the catabolism of body proteins would be reduced and the accumulation of organic acids could be kept at a minimum. Other investigators[6,19] have shown that a decrease in the blood sugar concentration and a rise in the concentration of glucose in the lavage fluid occurs if glucose is not present in the lavage fluid. This could be easily prevented by giving adequate amounts of dextrose.

The results obtained in the control dogs with Hartmann's and "A" solution were essèntially comparable in regard to the status of the fluid and acid-base balance. Certain advantages, however, may be accredited to "A" solution, namely, (1) it supplies more of the electrolytes commonly found in plasma than does Hartmann's solution, and (2) there is more bicarbonate available in the "A" solution.

The question of whether hormones were lost by such a procedure was considered, but since we have no information on this no positive statements can be made. It is felt, however, that animals and patients could probably combat the moderate deficits that might result.

Summary. From our studies and from previous work[6,11,12,19,25] it would seem that peritoneal lavage if properly employed for the treatment of uremia (and possibly other toxemias) could be an advantageous procedure.

It seems desirable to use the intermittent injection and withdrawal of a solution which has a chemical composition similar to that of interstitial fluid and is made slightly hypertonic by the addition of small amounts of dextrose (or gelatin or pectin). It would be essential to follow the acid-base balance closely by fairly frequent chemical determinations (total base, CO_2 content, plasma chloride level and pH), and in the event the procedure was carried out for any length of time, it would seem important to administer small

amounts of whole blood occasionally to prevent a marked depletion in the red blood cell and plasma protein concentration. If the electrolyte composition of the blood became abnormal it would be desirable to alter the composition of the lavage fluid as advocated by Rhoads[19] in order to remove solutes which were present in excess or provide ones which were decreased.

As has been emphasized previously[1,2,3,15] the hematocrit, hemoglobin, red blood cell count and plasma protein concentration would not provide reliable indices of an anemia or hypoproteinemia. Neither such studies nor the plasma electrolyte concentrations would be of much value in determining quantitatively the patient's state of hydration. The former problems could be controlled by the giving of an adequate diet or transfusions, while the latter could best be judged by careful and frequent observations of the patient, and the keeping of accurate intake and output records.

Conclusions. 1. In control animals urea nitrogen will diffuse quite rapidly into fluid which has been injected intraperitoneally.

2. A solution which has a chemical make-up similar to interstitial fluid would be suitable for lavaging the peritoneal cavity and would not greatly alter the state of hydration* or the acid-base balance.

3. Relatively large amounts of nitrogenous waste products and presumably other toxic elements can be removed from dogs by peritoneal lavage, keeping nephrectomized animals in relatively normal state for over a week.

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* In several human cases which were in a terminal state, the injection and withdrawal of such a solution has been tried. Although our experience in human patients is very limited, it would appear that the withdrawal of the fluid could be best accomplished by the use of a trochar or insertion of a rubber catheter, since the insertion of a needle does not, as a rule, permit a complete recovery of the injected fluid.

RELATIVE EFFICIENCY OF QUINACRINE (ATABRINE) AND QUININE IN TREATMENT OF ACUTE ATTACKS OF VIVAX MALARIA

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Prior to the introduction of quinacrine (atabrine) for the treatment of malaria, the medical profession and the public had, for centuries, accepted quinine without question as the best available drug for the treatment of malaria.

Quinacrine was introduced as a new anti-malarial drug in 1931 and was said to be effective in controlling acute attacks of malaria in 5 days during which time a total of 1.5 gm. had been administered by mouth. Early clinical trials in the field proved its value although it soon became apparent that the originally advocated regimen of 0.1 gm. three times daily for 5 days would have to be revised upwards so that not only would the total dose be greater but the amount given the first day also considerably increased, for prompt control of fever and symptoms especially in falciparum infections.

The curtailed supply of quinine as a result of war developments placed the major burden of malaria therapy on quinacrine. Extensive coöperative studies by military and civilian research groups on the pharmacology of quinacrine led to a more rational understanding of the relation of drug dosage, plasma level, and effectiveness in the treatment of acute attacks of malaria as well as clinical suppression. As a result of these studies specific recommendations for its use have been adopted with success by the military forces.

Nevertheless, the civilian medical profession has been reluctant to accept quinacrine freely and in many instances it has been prescribed grudingly only because quinine was not available. Numerous allegations of poor results are known for the most part to be the result of improper dosage.

The purpose of this paper is to present a comparison of the relative efficiency of quinine and quinacrine in the treatment of acute attacks of vivax malaria.

Treatment Schedules. A. *Quinacrine.* Three hundred and ninety-seven consecutive patients with acute clinical attacks of vivax malaria with fever and parasitemia were treated with quinacrine as follows: All treatment for purposes of uniformity was begun on the morning following the onset of the current attack. On this day referred to as Day 1 quinacrine dihydrochloride 4 tablets = 0.4 gm. was given after breakfast, 3 tablets = 0.3 gm. after lunch and 3 tablets = 0.3 gm. after supper making a total of 10 tablets or 1 gm. on the 1st day. On days 2 to 7 inclusive, 1 tablet of 0.1 gm. was given 3 times daily after meals so that a full course consists of 2.8 gm. during 7 days of therapy. Parasite counts were done twice daily and continued until negative for 3 consecutive days. Plasma quinacrine levels were determined on the 2d and 8th days. Clinical response was followed by daily rounds at which time all signs and symptoms possibly related to malaria or quinacrine were entered on special study forms. The patients left the wards on the 8th day and 200 of them were followed at a convalescent and reconditioning area for 120 days or until relapse. During this interval smears were examined twice weekly and in the event of parasitemia the temperature was recorded 4 times daily until the next smear. A temperature rise of over 100° F. by mouth associated with a positive smear was considered a relapse and the patient was readmitted to a ward for further observation and retreatment.

B. *Quinine.* One hundred consecutive clinical attacks of vivax malaria were treated

with quinine as follows: As with the quinacrine group, all treatment was started on the morning after the onset of the current attack. On Day 1, quinine sulphate in disintegrating tablets was given at exactly 8-hour intervals. Each dose was 1 gm. so that 3 gm. were administered on the 1st day. On Days 2 to 14 inclusive, 0.65 gm. were given 3 times daily at 8-hour intervals so that a total of 2 gm. were administered daily for each of the 13 days. Thus, a course of treatment consisted of the administration of 29 gm. of quinine sulphate during 14 days. Parasite counts were done twice daily until negative in thick drops for 3 consecutive days. Minimum and maximum plasma quinine determinations were performed on specimens obtained on the 2d, 7th, and 14th days of treatment. Clinical response was determined by daily rounds at which time all signs and symptoms possibly related to malaria or quinine were recorded on special study forms. The patients left the wards on the 15th day and were followed in a convalescent area for 120 days or to clinical relapse. In the interval, smears were examined twice weekly and in the event of parasitemic relapse the patient's temperature was recorded 4 times daily until the next smear. If a rise over 100° F. by mouth occurred associated with a positive smear the patient was admitted to the wards as a clinical relapse for retreatment and study.

Results. A. *Control of Parasitemia.* The rate of disappearance of parasites from the peripheral blood during administration of quinacrine and quinine, and their relative efficiency in this respect is shown in Chart 1. Within 12 hours after the first dose of quinacrine some patients already had negative smears. This did not occur with quinine. At 24 hours, 26% of patients receiving quinacrine were parasite free, but only 7% receiving quinine. At 48 hours the percentages were 77 and 44, respectively. At 72 hours only 4% of patients receiving quinacrine still showed parasites, and all were clear at 96 hours. With quinine, on the other hand, almost one-fourth still had parasitemia, and in a few, this persisted as long as 132 hours. Thus, in rapidly clearing the peripheral blood of malarial parasites, quinacrine is obviously significantly superior to quinine.

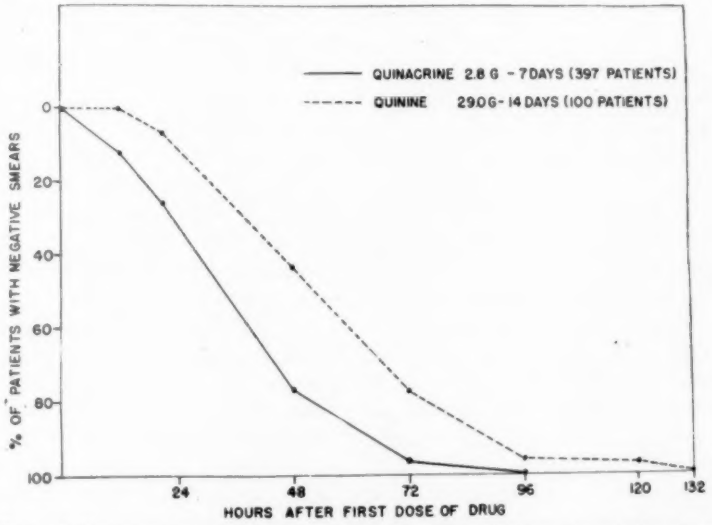

CHART 1.—Rate of disappearance of parasites during treatment of 497 acute attacks of vivax malaria with quinacrine or quinine.

B. *Control of Fever.* Of the quinacrine treated patients, 7% had a temperature of 100.2° F. by mouth or higher on the 2d or 3d days of treatment, and 10% of the quinine treated group had fever using the same criteria. While there is no significant difference between both drugs in their

effectiveness in controlling fever, the contention frequently advanced that quinine should be used for treatment during the first few days of an acute attack because of its superiority in controlling fever quickly is not borne out in this study. Further, in the treatment of delayed primary attacks of vivax malaria, 32% of patients who received quinine had fever on the 2d or 3d days after the beginning of treatment, while only 16% of delayed primary attacks treated with quinacrine had fever on the 2d day or subsequently after treatment was begun. Thus, quinacrine is more effective than quinine in controlling the fever of a delayed primary attack and is just as effective as quinine in controlling the fever of relapses.

C. *Control of Symptoms.* It is difficult to evaluate data on such symptoms as headache, backache, nausea, malaise, and weakness which are usually present for a few days in a treated attack of malaria in relation to the effect of any drug therapy in controlling them. Statistically there is little difference in the duration of these symptoms during treatment with quinine or quinacrine. However, one gains the clinical impression that these symptoms, particularly the weakness, are more strikingly and more promptly controlled during treatment with quinacrine than with quinine. The patients are anxious to get out of bed sooner, leave less food on their trays, and complain less of weakness or other symptoms during treatment with quinacrine than those receiving quinine. The latter remain in bed longer, seem more apathetic, and eat poorly in comparison to the quinacrine treated patients. Vomiting, abdominal pain and abdominal tenderness are controlled equally well with either drug.

On the whole it is the feeling of all the ward officers concerned in the management of these patients that quinacrine is more effective in promptly controlling the symptoms of an acute attack of malaria than quinine.

D. *Toxicity.* No major toxic manifestations were encountered during the administration of quinacrine. Patients who were reluctant to take quinacrine because of alleged previous intolerance were given the drug in colored capsules without their knowledge of the contents and responded well without symptoms of any kind. Persistent vomiting associated with the malaria attack was controlled by withholding fluids and food by mouth and by intravenous glucose prior to the administration of quinacrine. When the drug was given after several hours freedom from vomiting, this did not recur.

Central nervous system signs or symptoms were not encountered in relation to quinacrine therapy in this series. Two patients with severe eczematoid dermatitis suffered a flare up of their skin disease during quinacrine therapy. However, several patients with eczematoid dermatitis and acute malaria not receiving specific antimalarial therapy likewise had flare ups of the skin process and many others with eczematoid dermatitis and malaria suffered no ill effects from quinacrine. Nevertheless, it is our belief that patients with eczematoid or exfoliative dermatitis and acute malaria should not receive quinacrine if they have had this drug before.

Gastrointestinal symptoms controlled as previously described were not aggravated by quinine. Patients with eczematoid or exfoliative dermatitis and malaria had no activation of the skin process attributable to quinine. One patient receiving quinine developed acute thromopenia, purpura, and severe angioneurotic edema of the face on the first day of treatment. This patient was subsequently found to be extremely hypersensitive to quinine. Such a reaction though not unknown is admittedly very uncommon. A fairly high proportion of the quinine treated patients complained of severe and annoying tinnitus, buzzing, and fullness in the ears or head. In many instances this seemed to retard prompt and full recovery from the symptoms of the acute attack.

On the basis of possible toxicity related

to quinacrine or quinine the only points in favor of either drug are the absence of tinnitus with quinacrine. With either drug rare cases of idiosyncrasy or sensitization may be encountered.

E. *Effect on Relapse Rate and Interval to Relapse After Treatment.* Approximately 80% of Pacific infections relapsed within the period of observation of 120 days, whether treated with quinacrine or quinine. Thus, neither drug has any advantage with regard to the subsequent relapse rate as measured by 4 months' observation.

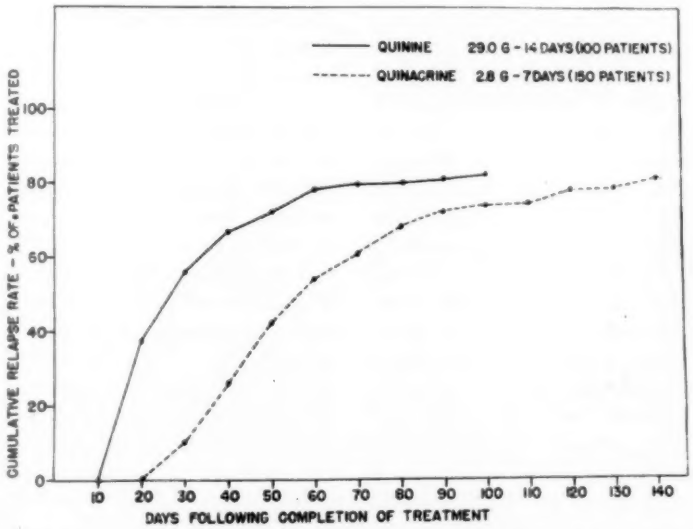

CHART 2.—Relapse rates and intervals to relapse following treatment with quinacrine or quinine of 250 acute attacks of vivax malaria of Pacific origin.

On the other hand there is a striking difference in the interval to relapse following treatment of acute attacks of vivax malaria with quinacrine or quinine. The cumulative relapse rates of the patients treated and observed to relapse or for 120 days as well as the interval to relapse is shown for both drugs in Chart 2.

Relapses following treatment with quinine begin as early as 12 days after completion of treatment and occur frequently during the first 30 days so that at the end of a month 55% of the entire group have relapsed. On the other hand following treatment with quinacrine, few relapses occur during the first 30 days and none in less than 21 days. At the end of a month only 9% of the treated patients relapsed compared to 55% of the quinine treated patients. At 40 days the relapses are 25% and 67%, respectively, for quinacrine and quinine. In other words at 40 days, 87% of the patients finally observed to relapse after quinine have done so while only 30% of those finally observed to relapse after quinacrine have done so in the first 40 days after treatment. At 60 days the maximum observed relapse rate of 80% in the quinine treated group has been reached, whereas following quinacrine the maximum observed relapse rate of 80% was not closely approached until 90 days and not actually reached until 120 days. This means that 30% of the observed relapses after quinacrine occurred after 60 days, while practically no relapses were observed later than 60 days after quinine. The mean interval to relapse following quinacrine is 53 days compared to 22 days following quinine.

Since neither drug materially influences the relapse rate, it is desirable to select a regimen which produces the longest pos-

sible interval to relapse. In this respect quinacrine is obviously very much better than quinine not only in effecting a longer interval to relapse by at least a month, reducing to a minimum relapses within 30 days, and providing in at least 30% of relapses an interval longer than 60 days.

Conclusions. 1. Quinacrine is superior to quinine in the treatment of acute attacks of vivax malaria for the following reasons: (*a*) More prompt and effective control of parasitemia and symptoms; (*b*) decidedly longer interval to relapse following completion of treatment; (*c*) absence of disturbing symptoms of cinchonism.

2. Quinacrine given according to the dosage plan described is more effective than quinine in controlling the fever of delayed primary attacks and is as effective in promptly controlling fever in relapses.

QUALITATIVE CHANGES IN FIBRINOGEN WHICH INFLUENCE THE ERYTHROCYTE SEDIMENTATION RATE AND THE CLOT RETRACTION TIME

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The work reported in this communication was carried out in an effort to find the ingredient or property inherent in plasma responsible for its ability to determine the blood sedimentation rate.

The blood sedimentation rate is essentially an expression of the degree of erythrocyte rouleau formation or clumping. Large clumps of cells will fall more rapidly than smaller ones. Although the aggregates are not spherical, Stokes' Law of Hydrodynamics, which deals with the rate of fall of spherical bodies in a liquid, depending upon their diameter, roughly applies.[1] If the actual process of sedimentation of blood in a hemocytometer is watched through a microscope tipped to a horizontal position, the large clumps of cells will be seen to fall rapidly, the single erythrocytes will fall slowly and at practically the same rate regardless of the sedimentation rate of that particular specimen of blood plasma, unless their speed is temporarily altered by eddy currents such as are produced by nearby rapidly falling clumps of cells.

Blood sedimentation rates are due to constituents of, or properties inherent in, plasma.[1] The erythrocytes play merely a passive rôle. Considerable investigation has been carried out in an effort to determine why two different plasmas of practically identical physical and chemical characteristics should cause widely different erythrocyte sedimentation rates. The yield of factual results has not been great.

If blood is deprived of its fibrinogen by clotting, the resulting serum will have lost whatever ability the plasma had to produce erythrocyte rouleau formation. This fact indicates that fibrinogen is the ingredient in blood that determines the erythrocyte sedimentation rate. Investigators have failed, however, to demonstrate any consistent relationship between the concentration of blood fibrinogen and the blood sedimentation rate. In these investigations, fibrinogen was presumed to be of the same composition in all bloods.

The ingredient in plasma sought for was found to migrate to the positive pole when plasma was subjected to an external field of electrical force. It failed to pass through a semi-permeable membrane, such as cellophane interposed in the field. These findings point to a large negatively charged molecule as the ingredient sought; any of the blood proteins may answer this description.

Fibrinogen of plasma samples was extracted by the common salting-out technique and redissolved in physiologic saline solution. Washed erythrocytes were found to manifest essentially the same degree of stability in these fibrinogen solutions as they did in the blood plasmas from which the fibrinogen samples were extracted. This finding more definitely points to fibrinogen as the constituent of plasma that determines the blood sedimentation rate. Although such evidence is confirmatory, it is not conclusive. Therefore, it was decided to find and study a simpler suspensoid which might simulate the blood sedimentation reaction. It was thought that examination of such a suspensoid might reveal facts that would lead to a more thorough understanding of the blood sedimentation reaction. An outline of the steps taken in the work that led to the opinions herein submitted may be of interest.

Procedure. A suspension of washed cells of commercial compressed yeast in a colloidal solution of gelatin was found to be a simple mixture that could be made to mimic the blood sedimentation reaction. The hemagglutination of erythrocytes by gelatin was

described in 1920 by Karsner and Hanzlik.[4] An increase in the blood sedimentation velocity following the intravenous use of gelatin solutions therapeutically has been reported.[7]

Blood sedimentation velocities were simulated by packed cell volumes of 10 to 33% yeast cells in 0.25 to 5% aqueous solutions of gelatin. The rate could be diminished by diluting the suspensoid with water. It could be accelerated by increasing the temperature of the solution or by tipping the containing test tube from the perpendicular, as is the case when sedimenting blood is subjected to these influences.[6] The yeast sedimentation rate could be rechecked after shaking the tube enough to resuspend the cells. The possibility of specific gravity being an important factor in the process was discredited in light of the fact that the addition of gelatin to water increases its specific gravity, but also increases the solution's ability to produce a rapid sedimentation of yeast cells.

Yeast cells lend themselves well to this type of experimental work, because they are able to withstand relatively wide excursions of osmotic pressure, temperature, and pH changes. Sedimentation rates of yeast cells in plasma samples were slightly slower than, but paralleled those of erythrocytes. The yeast used was commercial compressed yeast or Saccharomyces Cerevisial Hansen.[5] The cells have a density of approximately 1.0 to 1.1; they are oval in shape, and quite variable in size, measuring from 5 to 7 microns on the short axis, and from 7 to 9 microns on the long axis. They carry a negative electric charge in aqueous suspension. Red blood cells carry a negative charge in plasma.[1]

In the first series of experiments, solutions of commercial gelatin were used as the dispersing phase. The degree of sedimentation of yeast cells or erythrocytes was found to be directly proportional to the concentration of gelatin in the suspending solution, up to about 5%. Greater concentrations of gelatin were too viscous, and jelled too rapidly to be tested.

Commercial gelatin is commonly a mixture of two types of gelatin.[8] One type is prepared from an acid-treated precursor, to yield a gelatin positively charged in aqueous solutions, its pH is about 4.0, its isoelectric point is pH 7.8 to 9.3. The second type is prepared from an alkaline-treated precursor, to yield a gelatin negatively charged in aqueous solutions. Its pH is about 6.0, its isoelectric point is approximately 4.7. The hydrophilic properties of both of these gelatins are dependent on pH, and also the pH in relation to the isoelectric point. For instance, the first type shows a minimum water absorption at pH 8.0, and its ability to absorb water increases on either side of this point. The second type shows a minimum water absorption at pH 4.7, and its ability to absorb water increases with changes away from this, its isoelectric point. Samples of each of these two types of gelatin were secured from a reliable concern.

Yeast cells sediment rapidly in aqueous solutions of negatively charged gelatin, but very slowly in aqueous solutions of positively charged gelatin (Fig. 1). The same reaction was noted when erythrocytes were substituted for yeast cells, and physiologic saline for water (Fig. 2).

The difference in sedimentation velocity of the cells in the two gelatin solutions was at first thought to be due to the difference in electrical charge carried by the gelatin. Further experimentation proved that the pH of the solution, rather than the type of charge carried by the colloid, was responsible for the difference. Solutions of negatively charged gelatin caused little sedimentation of yeast cells in a solution at a pH of 4.7, which is its isoelectric point, but a very rapid rate at a pH of 8.0. Positively charged gelatin reacted similarly in relation to its isoelectric point. The rate of sedimentation of yeast cells was found to be directly proportionate to the degree of water absorption of gelatin at the pH of the solution in question. The larger the amount of unbound water, the greater the stability of the suspensoid, indicating that the individual cell may require a certain amount of unbound water to maintain suspension.

The pH of each of a series of tubes of 25% yeast in a 2% solution of negatively charged gelatin, was adjusted to represent the greatest range of pH the cells could tolerate. After standing for 1 hour the amount of cellular sedimentation in each tube was noted, and the results recorded

in graphic form. The degree of sedimentation was not uniformly progressive with changes in pH. The graphic result was a series of waves increasing in amplitude from the pH range producing the least to that producing the greatest sedimentation velocities. Erythrocytes could not be subjected to such testing because of their intolerance for pH changes.

This finding suggested the possibility that gelatin may be composed of fractions which differ in the way they affect the sedimentation of yeast cells at a given pH. Therefore, gelatin was fractionated by the common salting-out technique. The various fractions were found to differ considerably in physical and chemical characteristics. It is not within the scope of this paper to point out in detail the differences in the various fractions of gelatin. Suffice it to say that they differ in consistency, texture, pH, isoelectric point, solubility in

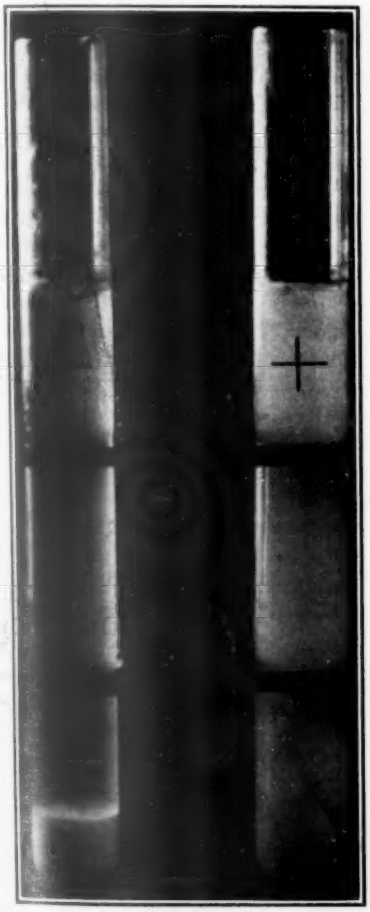

FIG. 1

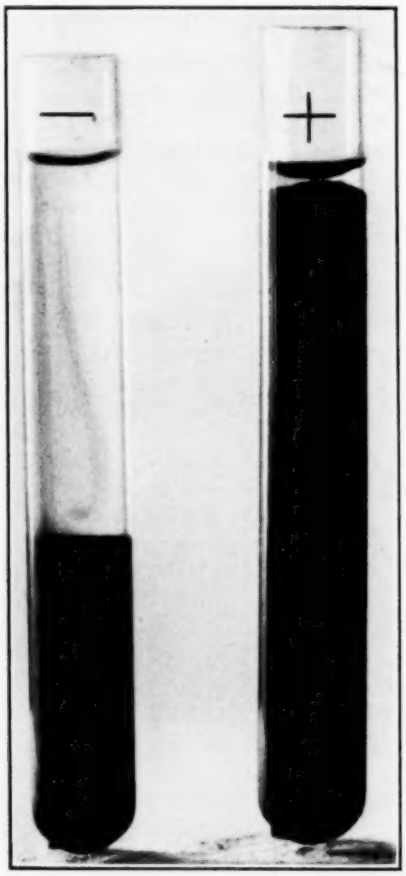

FIG. 2

FIG. 1.—Each tube in this photograph contains a 10% packed cell volume of yeast cells suspended for 30 minutes in a solution of 2% gelatin in water. The gelatin in the tube to the left carries a negative charge; that in the tube to the right bears a positive charge.

FIG. 2.—The tube to the left is of 40% packed cell volume of washed erythrocytes in a solution of 2% negatively charged gelatin in physiologic saline. The tube to the right is of the same composition except that the gelatin in this tube bears a positive charge. Photograph was taken 30 minutes after tubes were set up.

water, solubility in relation to temperature, ability to produce aggregates of yeast cells or erythrocytes, and optimum pH required for clumping of these cells. The first fractions to be salted-out precipitated as a rubber-like, gummy, tenacious mass. When redissolved, these fractions were capable of producing a rapid sedimentation of yeast cells or erythrocytes. Those fractions, which were salted-out only by greater concentrations of saturated solution of ammonium sulfate, produced a precipitate which was flaky, rather than flocculant. On redissolving, they exhibited but slight ability to cause erythrocyte, or yeast cell clumping.

The finding that gelatin is composed of certain fractions which are able to induce erythrocytes to form aggregates, and other fractions which are relatively inert in this respect, led to the assumption that blood fibrinogen may be similarly composed. Because its plasma produces a rapid sedimentation rate, the fibrinogen of women in the last trimester of pregnancy was studied. It was found to resemble gelatin in that it, too, could be fractionated by the usual salting-out technique. It also

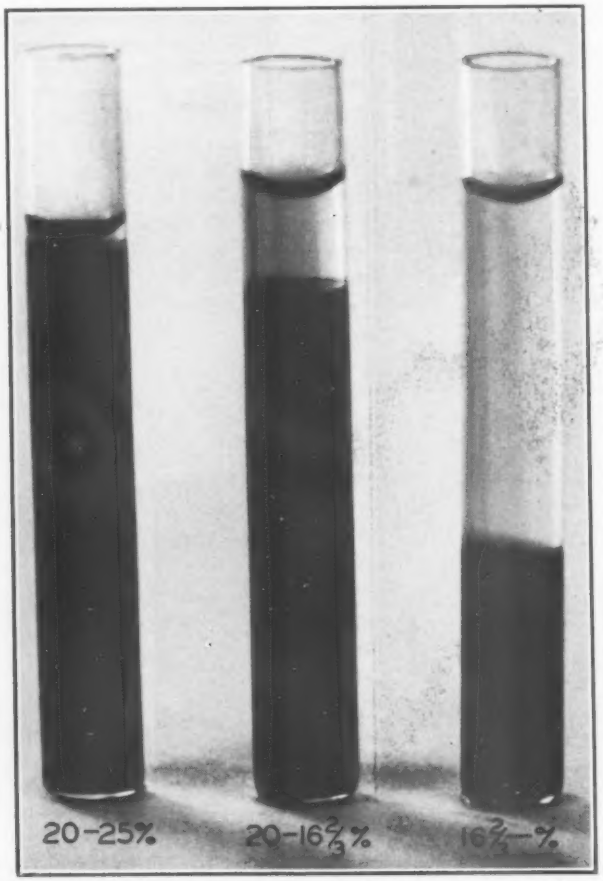

FIG. 3.—Fibrinogen from a single blood sample was fractionated into 3 parts by the common salting-out technique. Each fraction was redissolved in sufficient physiologic saline to make a 0.1% solution. Four cc. of washed erythrocytes were suspended in 6 cc. of each of the fibrinogen solutions. This photograph was taken 30 minutes after the tubes were set up. They show the influence of various fractions of fibrinogen upon the sedimentation rate. The figures below the tubes indicate the percentage of saturated solution of ammonium sulfate employed to precipitate out each of the fibrinogen fractions.

was found to be composed of fractions which were very active in causing a rapid erythrocyte sedimentation rate, and other fractions which were relatively inert in this respect (Fig. 3). Like gelatin, the fractions salted-out by the least amount of saturated solution of ammonium sulfate, caused a relatively flocculent precipitate which settled as a tenacious, rubbery mass. These fractions were found to be the ones most active in producing a rapid erythrocyte sedimentation velocity. One part of a saturated solution of ammonium sulfate to five parts of plasma, produces a solution $\frac{1}{6}$, or $16\frac{2}{3}$%, of which is the salting-out solution. Ordinarily no great amount of blood protein is expected to be separated out of plasma by such a low concentration of this salting-out agent. However, at this concentration there is a considerable amount of blood protein separated out in those plasmas which produce a rapid erythrocyte sedimentation velocity. In our laboratory this group of proteins was called "contractinogen," because of certain physical characteristics which will be subsequently described. For convenience of expression, they will be referred to as such in this communication. The clot formed by contractinogen will be referred to as "contractin." It is not to be inferred that these are newly-discovered blood proteins, but rather that they are part of a "fibrinogen complex" which may assume qualitative changes during pregnancy and in certain disease conditions.

Contractinogen is the first of the blood proteins to be salted-out upon the addition of a saturated solution of ammonium sulfate to blood plasma; next comes the remaining portion of the fibrinogen complex and so forth. In bloods which manifest a rapid blood sedimentation rate contractinogen may constitute as much as a third of what is ordinarily considered fibrinogen. The amount is smaller in those bloods which manifest a slower blood sedimentation velocity. In a series of 90 blood samples, the amount of contractinogen was found to be directly proportionate to the erythrocyte sedimentation velocity.

Contractinogen precipitates out when plasma is subjected to cooling. The amount of precipitate depends on the amount and type of contractinogen in the plasma, and the degree of cooling; this reaction is reversible. The plasma of a

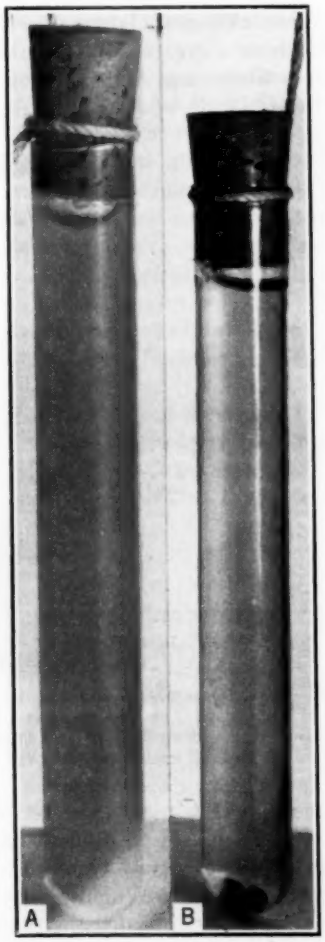

FIG. 4A.—Fibrinogen which was salted out by a 16.7% solution of saturated solution of ammonium sulfate was redissolved in water and allowed to clot. The photograph is of a test tube nearly filled by the newly formed clot which has not yet begun to retract.

FIG. 4B.—This photograph, taken 5 minutes later, is of the same clot shown in Figure 4A. In the interim the clot had been freed from the side walls of the test tube and allowed to retract down to the small, dark mass seen in the bottom of the tube.

blood with a rapid sedimentation rate will become turbid and slowly form a precipitate of contractinogen at near freezing temperatures. This insolubility could account for the influence of temperature upon the blood sedimentation rate.

Contractinogen clots as a delicate white elastic mantle. In an aqueous solution in which the process is not impeded by blood cells, contractinogen clots more rapidly and manifests a greater ability to retract than does fibrinogen. Upon being freed from the side walls of the containing test tube, such a clot may contract down within a few minutes to a small part of its original volume (Figs. 4*A* and 4*B*). The presence of a soft, frail, mantle-like contractile clot may be demonstrable in bloods with a fast sedimentation rate which are diluted beyond their ability to form a definite fibrin clot.

Contractinogen gradually clots out of blood plasma upon standing *in vitro*, in spite of the presence of such anticoagulants as oxalates and citrates. This may help to explain why the blood sedimentation rate becomes slower after blood has been allowed to stand for several hours. It also suggests a difference in the mechanism of clotting of contractinogen and fibrinogen. Experimental evidence was accidentally found which supports the opinion that various fibrinogen fractions reveal different clotting characteristics. Blood fibrinogen may be shown to clot not just once, but rather to produce a succession of clots. A successful demonstration of these phenomena requires that each successive clot be allowed to retract soon after it is formed (Fig. 5). This work will be reported in a subsequent paper.

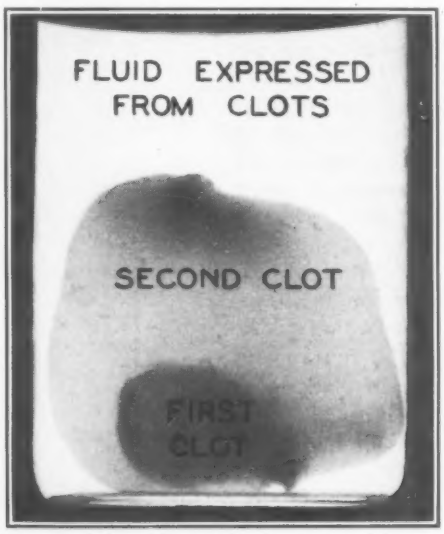

FIG. 5.—This photograph is of a clot within a clot. Each clot was formed from the same aqueous solution of fibrinogen. The first clot was separated from the side walls of the test tube and allowed to retract soon after it formed. The clear fluid expressed from the clot formed slowly into a second clot. The second clot, as did each succeeding one, revealed decreasing ability to retract.

Hirschboeck and Coffey, in a study of the clot retraction time in thrombophlebitis and pulmonary embolism, noted a correlation between the rapidity of clot retraction and erythrocyte sedimentation.[3] They inferred from a report by Ham and Curtis[2] that the erythrocyte sedimentation rate was chiefly dependent on the fibrinogen level of the blood plasma. This is a view not shared by most investigators in this field. In their opinion, the increase in the amount of fibrinogen led to an increase in

the fibrin matrix of the clot. They considered this responsible for a more rapid and forceful retraction. I concur in the opinion that those bloods with a rapid erythrocyte sedimentation rate may be expected to demonstrate a rapid clot retraction time. It is my opinion that qualitative, rather than quantitative changes in the fibrinogen are the basis for this behavior. Accordingly, a rapid erythrocyte sedimentation rate or a fast clot retraction time indicates a shift of the fibrinogen complex toward those components which are most readily salted-out of plasma upon the addition of saturated solution of ammonium sulfate.

Summary and Conclusions. 1. A suspension of yeast cells in aqueous gelatin solutions may be made to mimic the erythrocyte sedimentation reaction.

2. The yeast cell sedimentation rate in gelatin solutions is dependent upon the composition and concentration of the gelatin; the electrical charge the gelatin carries; and the pH of the solution.

3. The findings, as set forth in this paper, might well be used to advantage to combat the production of rouleau formation following the therapeutic use of gelatin solutions intravenously.

4. Certain fractions of gelatin are responsible for its ability to produce a rapid sedimentation of yeast cells or erythrocytes. These fractions are the first to be salted-out upon the addition of a saturated solution of ammonium sulfate to a gelatin solution.

5. Fibrinogen may be divided into component parts by the common salting-out technique. These component parts display unlike physical characteristics.

6. The fibrinogen fractions first to be salted-out upon the addition of saturated solution of ammonium sulfate to plasma are the ones most active in producing a rapid erythrocyte sedimentation rate. These fractions precipitate out upon cooling and gradually clot out upon standing, even in the presence of anticoagulants.

7. Fractions of fibrinogen differ in clotting characteristics.

8. The fractions of fibrinogen which determine the erythrocyte sedimentation rate are probably the same ones which determine the speed and degree of clot retraction.

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PERSISTENT VENTRICULAR BIGEMINAL RHYTHM IN APPARENTLY NORMAL HEARTS

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Premature contractions, or extrasystoles, may arise in various parts of the heart; the auricles, ventricles, or various points in the conduction system. For the most part, their occurrence is not significant, and in themselves premature beats do not indicate an abnormality in the heart. In some individuals premature beats may occur so infrequently that they are never appreciated by the individual, whereas in others they may be so frequent and persistent that they may become a source of anxiety and at times may be incapacitating by virtue of the symptoms and anxiety they cause.

The cause of premature beats of the heart is not known. Over-exertion, excitement, emotional stress and strain and fatigue are factors often related to their occurrence. Coffee, tea, alcohol, tobacco and certain drugs, for example, digitalis, may also produce premature beats. Experimentally, they may be produced by direct mechanical or chemical stimulation of the heart or by stimulation of the autonomic nervous system. They may be produced by vagal stimulation, either directly or reflexly or by stimulation of the sympathetic division of the autonomic nervous system. Allen[1,2,3] has produced premature ventricular beats resulting in bigeminal rhythm in rabbits by mechanical stimulation of the nasal septa and has shown that the stimulus is transmitted through the sympathetic nervous system. The work of Beattie and Brow (cited by Levine[5]) indicates the presence of a center in the brain that is concerned with the formation of premature beats of the heart. It is clear then that while a number of facts are known about premature beats, the actual mechanism of their production is unknown. There is some evidence for a structural neurogenic basis for them.

When a premature ventricular beat regularly follows each normal contraction, a bigeminal or coupled rhythm results. The commonest cause of such a rhythm is overdosage of digitalis. In other instances it may be due to organic heart disease. It may occur when premature beats arise in the auricles or the auriculoventricular node as well as when they arise in the ventricles. Rarely it may be caused by 3:2 heart block when every third auricular beat is blocked and fails to reach the ventricle. Finally, a persistent coupling of the rhythm due to premature ventricular beats may occur in apparently normal, healthy individuals in the absence of any recognizable cause. It is with these cases that this report is primarily concerned.

During the period from April 1, 1941, through October 1, 1944, a total of 3754 electrocardiograms were made in the heart station of the North Carolina Baptist Hospital in Winston-Salem, North Carolina. An electrocardiographic diagnosis of bigeminal rhythm was made in 33 instances. In 31 of these cases the bigeminal rhythm was due to premature ventricular beats; in 2 it was due to premature auricular beats. In 12 of the 33 patients the bigeminal rhythm occurred in the presence of well marked heart disease; in 13 patients it had been induced by digitalis overdosage; while in 8 patients the arrhythmia occurred in the absence of organic heart disease or of digitalis (Table 1). These patients are of particular interest, since none of the usual causes of bigeminal rhythm were present.

Four of the 8 patients in whom evidence of heart disease was lacking had very frequent, irregularly occurring premature beats, and the coupled rhythm occurred only intermittently and in short runs. These 4 patients exhibited no positive evidence of heart disease, although its absence could not be stated with certainty, since 3 of them were past 67 years of age, and all had considerable arteriosclerosis.

Table 1.—The Causes of Bigeminal Rhythm in 33 Cases

Underlying cause	No. of cases
Organic heart disease	12
Digitalis	13
No demonstrable cause	8
Total	33

In 2 patients, careful examination seemed to eliminate the presence of organic heart disease. Here again the premature beats came frequently and irregularly, with only occasional short periods of bigeminal rhythm. Both patients were females in whom profound emotional factors were operating. In 1 patient the premature beats completely disappeared after the fundamental emotional disturbance was controlled. The other patient did not respond well to psychotherapy, and at the time of discharge from the hospital, premature beats were still occurring rather frequently, although the bigeminal rhythm had not recurred.

Finally, there were 2 patients who exhibited a persisting *bigeminal*, or coupled, rhythm. Neither of these patients had heart disease nor any known cause for the arrhythmia. The first was a 12 year old mentally deficient girl. She had been admitted to the hospital because of congenital nystagmus and a congenital web of the larynx. The bigeminal rhythm was due to premature ventricular beats. Physical examination revealed no other cardiac abnormalities. The electrocardiogram, except for the bigeminal rhythm, was normal. This patient was observed over 3 months, and the bigeminal rhythm was present at all times. The second patient was a 33 year old woman with rheumatoid arthritis. There was no evidence of heart disease. The only abnormality, aside from rheumatoid arthritis, was a well marked emotional disturbance related to the skeletal deformity and incapacity caused by the arthritis. During her 6 weeks hospital stay she had bigeminal rhythm with only occasional short periods during which the rhythm was normal. We have, then, observed 2 patients who have exhibited a constant bigeminal rhythm and in whom none of the usual causes of this arrhythmia could be discovered (Table 2).

Bigeminal rhythm is not usually thought of as occurring in normal individuals. The standard textbooks of heart disease[5,6,7] do not mention the possibility, and in the English literature we have been able to find but one reference to such an occurrence. Coogan[4] has described persistent bigeminal rhythm of 2 years' duration in a 38 year old female. This patient had been under considerable emotional stress and strain. The bigeminal rhythm and other symptoms disappeared immediately when the cause of the emotional disturbance was removed. Coogan also cited the case of a professor who had bigeminal rhythm for 10 months while he was writing a book. The arrhythmia disappeared immediately when the strain of writing was removed. There are a number of references in the foreign literature the titles of which suggest that this condition may occur more frequently than the references in the English literature indicate. Most of the foreign references are unavailable at the present time.

The cause for the occasional occurrence of persistent bigeminal rhythm due to regularly occurring premature ventricular beats in normal persons is unknown. No attempt was made to determine the path-

ogenesis in the patients reported here. Quinidine was given to 1 patient (T.B.) without effect on the occurrence of the bigeminy. Coogan's patient had a bradycardia, and he postulated that the bigeminal rhythm was due to ventricular escape from the slow sinus rate. Atropine given to this patient speeded the heart rate and abolished the premature beat for a period of 36 hours.[4] Most of the cases have occurred in females, and in most cases some emotional disturbance seemed to be an important factor. In those cases in which the bigeminal rhythm occurs only in short runs and is not persistent, it is probable that it is a chance occurrence incidental to the frequent, irregularly occurring premature beats. It seems unlikely, however, that this is true when the coupling is persistent. The necessity for a premature beat to *follow regularly each* normal contraction in order to produce the arrhythmia is evidence against this. Further study is necessary to determine the mechanism of this arrhythmia when it occurs in persons with normal hearts.

TABLE 2.—BIGEMINAL RHYTHM OCCURRING IN 8 PATIENTS IN THE ABSENCE OF DIGITALIS OR DEMONSTRABLE HEART DISEASE

Patient	Age	Sex	Rhythm	Associated conditions
A. Inconstant Bigeminy Occurring in Patients in Whom Heart Disease Could Not Be Definitely Ruled Out Because of Such Factors as Age and Arteriosclerosis				
M. B. E.	72	F	Frequent premature ventricular contractions with short, intermittent periods of bigeminy	Cerebral arteriosclerosis
M. A. B.	67	F	Same	Carcinoma of breast; benign adenoma of thyroid
F. H.	37	M	Same	Duodenal ulcer; pul. tuberculosis
J. O.	75	M	Same	Empyema; latent syphilis
B. Inconstant Bigeminy Occurring in Patients in Whom Heart Disease Could Be Definitely Ruled Out				
M. D.	48	F	Frequent premature ventricular contractions with short, intermittent periods of bigeminy	Anxiety state; menopause
T. L. T.	28	F	Same	Anxiety state; hyperven. tetany
C. Constant Bigeminy Occurring in Patients in Whom Heart Disease Could Be Definitely Ruled Out				
J. M. P.	12	F	Constant ventricular bigeminal rhythm	Mental deficiency; congenital web of larynx
T. B.	33	F	Persistent ventricular bigeminy with only rare and very short periods of normal rhythm	Anxiety state; rheumatoid arthritis

Conclusion. 1. Bigeminal rhythm due to premature ventricular contractions may occur in the absence of heart disease and in the absence of digitalis intoxication, or other detectable cause.

2. It occurs commonly in those individuals with irregularly occurring premature ventricular beats, in which cases the coupling of the beats usually occurs only in short, intermittent periods. Less commonly it may be constant and persist for weeks or months. That the latter may occur in individuals with normal hearts is a fact not generally recognized.

3. It occurs most frequently in females and is apparently related in most instances to some emotional disorder. Usually when the emotional disturbance can be eliminated, the arrhythmia will disappear.

4. The mechanism of production of the arrhythmia in otherwise normal individuals is unknown.

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FUNCTIONAL PAROXYSMAL AURICULAR FIBRILLATION

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The term uncomplicated or functional paroxysmal auricular fibrillation is used to describe the type in which no evidence of organic cardiac disease can be demonstrated. This condition occurs frequently and the literature on this subject has been well summarized by Parkinson and Campbell[9] and also by Orgain, Wolff and White.[8] This type of functional arrhythmia is frequently seen in Naval personnel. In a period of 3 weeks at this Naval Hospital, 5 patients were seen in consultation suffering from this variety of irregularity.

The purposes of this report are to emphasize the frequency with which the functional paroxysmal auricular fibrillation is observed in Navy personnel (and presumably in the Army) and to express our definite conviction that patients with this arrhythmia should be discharged from the Navy or retained for limited duty ashore only. This conclusion is contrary to the one implied in the many published reports[8,9,10] which emphasize the benign nature of the irregularity. Furthermore the lessons to be learned from this study are directly applicable to civilian practice.

The 5 case histories which follow present only the salient features.

Case 1. R. C. C., male, age 41, weight 142 pounds, height 65″. This patient had been on active duty in the Chaplain Corps, USN, for 2½ years. His illness began 4½ years before admission to this hospital. Suddenly, one evening, while he was delivering his usual Sunday sermon, he had become aware of palpitation and irregular beating of his heart. He felt rather faint and had to interrupt the service. Subsequently, an electrocardiogram was taken and he was told by the physician that he had an irregular heart. He was given some pills which he thinks were quinidine. The symptoms disappeared.

For the next 4 years he took very little medication, but was frequently annoyed by spells of palpitation of variable length. These were apt to occur at any time of the day or night; they usually began suddenly and ended abruptly and lasted from several minutes to several hours. Toward the end of this period, he had short attacks almost every day, but they were never prolonged or severe enough to interfere with his usual activities.

Late in August, 1944, he was on Saipan conducting burial services for battle victims. He had been working in the hot sun, standing continuously for 3 hours, when he noticed severe palpitation and felt very dizzy. He was forced to lie down in the shade. Recovery from this episode was prompt, but his medical officer ordered him to be evacuated. He arrived at the U. S. Naval Hospital, Navy No. 10 for further treatment.

On September 17, 1944, he was seen in consultation. The past history was negative for rheumatic fever, chorea, scarlet fever and other significant illnesses. The general physical examination showed a middle aged man, of average build, in no acute distress or discomfort; there was no dyspnea. Significant findings were limited to the cardiovascular system. The heart action was grossly irregular; the ventricular rate between 90 and 150 per minute; blood pressure was about 110/80, 110/88. Cardiac size and contour were normal. No murmurs were heard. The lungs were clear; there were no signs of congestive failure. Regular sinus rhythm was readily induced by small doses of quinidine, but frequent doses were necessary to prevent reversion to fibrillation.

It was ascertained that the attacks of palpitation were more apt to occur while the patient was lying supine, and symptoms were alleviated in the prone position. Standing seemed to accentuate the symptoms of palpitation and the patient usually became dizzy if he stood for a long time or exerted himself. His habits were temperate; he usually smoked about 20 cigarettes per day and drank but 1 cup of coffee. Although he

had experimented with the possible effect of these agents, he had been unable to establish any relationship.

During periods of regular rhythm, all studies were negative. The pulse rate was usually slow, about 60 beats per minute or slightly less. Roentgen ray examination of the chest was negative. Fluoroscopy of the heart indicated a normal size and contour with good pulsations. Basal metabolic rate was minus 5. The blood count was within normal limits. The Kahn test was negative. Sedimentation rate was normal. Psychiatric consultation resulted in a negative report.

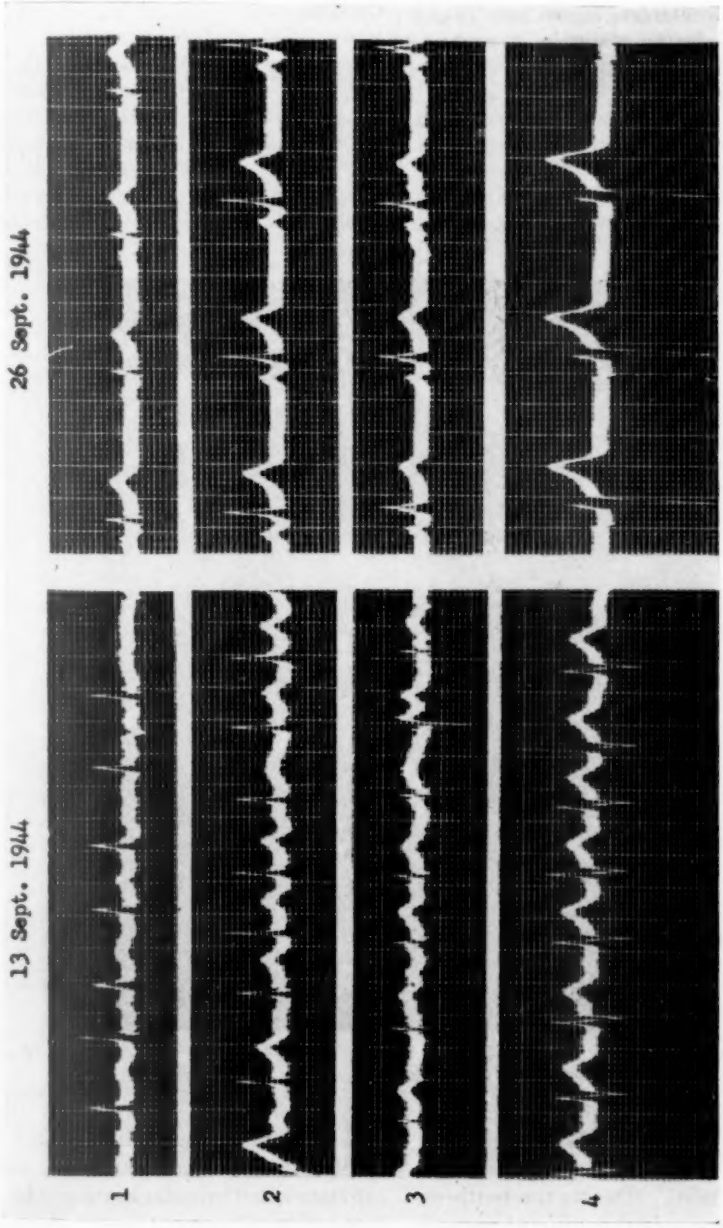

FIG. 1A.—(Case 1.) R. R. C., male, 41, C. C., USNH, Navy 10. Paroxysmal auricular fibrillation, September 13, 1944. Ventricular beat completely irregular, rate about 120 to 150 a minute, with an occasional premature ventricular systole. There is no abnormality of the QRS and T-waves; that is, no evidence of myocardial involvement. Regular sinus rhythm September 26, 1944, rate 60 64 a minute.

The control and the electrocardiograms after the "Master 2-step" exercise test were negative. In other words, there was no evidence of latent coronary insufficiency.

Without quinidine sulphate, the patient tended to have a daily reversion to auricular fibrillation. The effect of the quinidine was relatively transient and it was necessary to administer 4 or 5 tablets, each 0.2 gm. (3 gr.) per day in order to maintain a continuous regular sinus rhythm. Before evacuation the patient was able to get along with but 4 tablets a day. He was returned to limited duty in continental U. S. A., since it was concluded that his duties must not be arduous and that medical attention should always be available.

tating rest. No organic cardiovascular disease was present, and no cause for the arrhythmia was found. During the periods of regular rhythm the rate was slow and occasional premature ventricular systoles were observed. Quinidine sulphate was necessary to maintain a continuous sinus rhythm.

CASE 2. D. F. W., male, age 23, weight 170, height 5′ 11″. This patient was in the naval service for 2 years and had been able to perform his duties as an aviation machinist mate 2/c without any physical discomfort. The past history was negative for significant illnesses. Six weeks before admis-

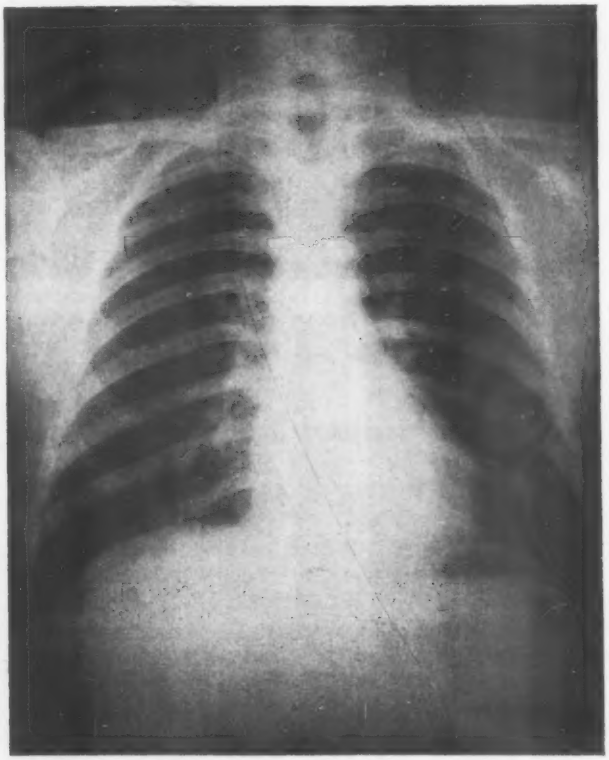

FIG. 1*B*.—(Case 1.) R. R. C., male, 41, C. C., USNH, Navy 10. Paroxysmal auricular fibrillation. Teleoroentgenogram discloses normal heart and lungs.

Summary. A chaplain of 41, experienced almost daily attacks of paroxysmal auricular fibrillation. During the battle on Saipan a severe episode occurred necessi-

sion to the hospital, while engaged in his usual duties, he became suddenly aware of palpitation and irregular beating of his heart. During the following 2 weeks, these symp-

toms persisted but he continued at his work, although he avoided strenuous exertion. He sought medical advice and was turned into the sick bay. Shortly thereafter, he was transferred to this hospital. On admission, he did not appear acutely ill. The general physical examination was negative except for the following findings: the heart beat was absolutely irregular; the apical rate was 104 per minute, the radial pulse was 84 per minute; that is, a pulse deficit of 20. The blood pressure was 98/58. There was no enlargement of the heart. No murmurs were heard and the heart tones were good. An electrocardiogram indicated the existence of auricular fibrillation but no evidence of myo-

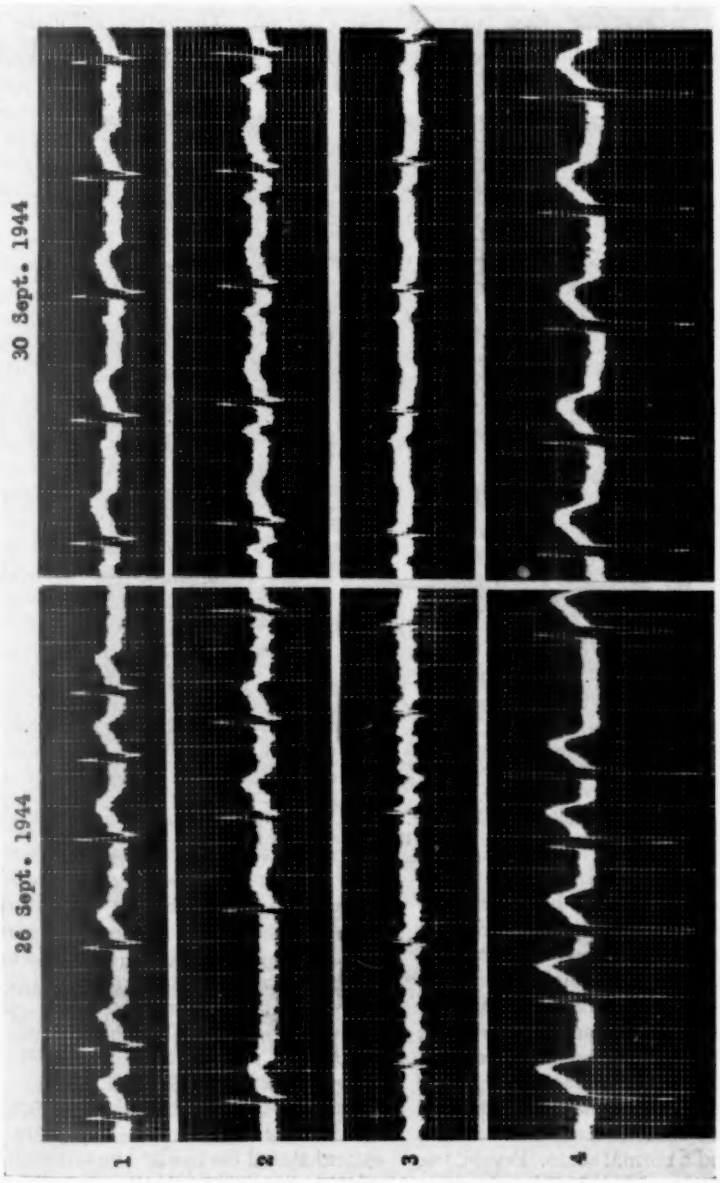

FIG. 2A.—(Case 2.) D. F. W., male, 21, AMM 2/c, USNH, Navy 10. Paroxysmal auricular fibrillation, September 26, 1944. Ventricular beats completely irregular, rate 50 to 150 a minute, with an average of about 100. There is no abnormality of the QRS and T-waves; that is, no evidence of myocardial involvement. Regular sinus rhythm September 30, 1944, heart rate 75 to 80 a minute.

cardial involvement was present; that is, there were no significant Q waves, the RS–T segments were isoelectric, and the T-waves upright. The patient was given quinidine sulphate, 0.2 gm. (3 gr.), 4 times a day. Two days later this was increased to 5 times a day, and after 2 days more it was given every 3 hours. After 48 hours of the latter dosage, the palpitation ceased abruptly and an electrocardiogram showed a normal sinus rhythm with no evidence of myocardial involvement. At this time the cardiac rate was 60 beats per minute, the blood pressure 120/72. Quinidine medication was discontinued and the regular sinus rhythm persisted. Complete studies including physical examination, electrocardiogram after "Master 2-step" exercise test, blood counts, sedimentation rate, and Kahn test were negative. The basal metabolic rate was plus 1. Fluoroscopy of the chest indicated normal cardiac size and contour, good contractions of the heart, and a normal aorta. Psychiatric consultation disclosed nothing significant.

No primary relationship could be established between the occurrence of the arrhythmia and such potential factors as body position, coffee or tea, or smoking.

Summary. A young man of 23 developed an attack of auricular fibrillation while performing his usual duties. While the arrhythmia was present he avoided strenuous exertion. Quinidine sulphate established regular sinus rhythm which was maintained when the drug was withdrawn. A bradycardia was noted during regular sinus rhythm. No evidence of any organic cardiovascular disease was ascertained and no precipitating factor (for the attacks) was proven.

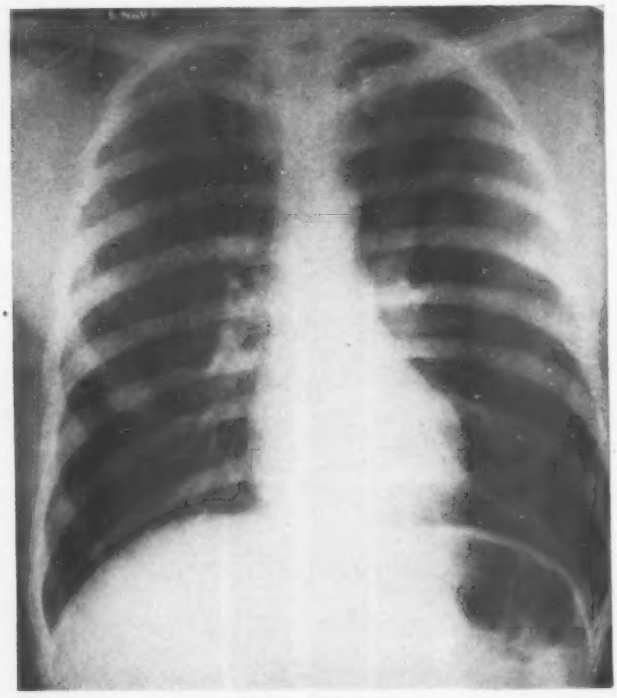

FIG. 2*B*.—(Case 2.) D. F. W., male, 21, AMM 2/c, USNH, Navy 10. Paroxysmal auricular fibrillation. Teleoroentgenogram September 17, 1944 discloses normal heart and lungs.

CASE 3. C. F. T., Lt. Comdr., age 48, on active duty for the past 3½ years. The patient stated that he had no serious illnesses prior to September, 1943. At this time, he

developed symptoms of acute appendicitis and at operation, a gangrenous appendix was removed. Surgical convalescence was uneventful. The day following operation, an irregular pulse was noted and subsequent investigation disclosed the presence of auricular fibrillation. He received digitalis but no additional facts were recorded in the health record. The patient gave the information that subsequent to this episode he experienced daily sensations of palpitation which were never severe enough to interfere with his duties. This palpitation would last from 15 minutes to 2 hours. On September 2, 1944, while at general quarters aboard a battleship, he had severe palpitation and collapsed. He is said to have been unconscious for about 5 minutes, and when seen by the medical officer he was clear mentally but appeared "ashen, pale and cyanotic." The pulse was completely irregular, rate about 150 per minute. The blood pressure was 50/0. On admission to this hospital, later on the same day, his only complaint was palpitation. The physical examination at the time of admission was said to have been entirely negative except for the irregular heart action. An emergency electrocardiogram was ordered but before this could be taken, the patient's rhythm had reverted to normal and the tracing was negative. It showed a regular sinus rhythm with no evidence of myocardial involvement. One week later he had another mild attack of palpitation but did not call the medical officer. On September 25, the irregularity recurred suddenly and an electrocardiogram made during the attack showed auricular fibrillation with occasional ventricular premature systoles. This attack terminated abruptly after several hours without the aid of medication. He was then placed on quinidine sulphate (0.2 gm.) twice a day as a prophylactic measure and did not have a recurrence of the fibrillation during the remainder of his stay in the hospital. When the rhythm was regular the pulse rate averaged about 56 per minute. The patient felt that while the attack was in progress he was more comfortable if he would lie flat on his back. He did not smoke, but chewed tobacco. He drank about 4 cups of coffee per day and partook moderately of beer.

Complete studies, including electrocardiograms after exercise, blood counts, sedimentation rate and Kahn tests were negative. Fluoroscopy of the heart showed normal size and contour with good pulsations and a normal aorta. BMR was plus 3. Medical discharge was recommended for this patient since it was obvious that a recurrence of the arrhythmia at a critical moment during the performance of his duties might jeopardize the safety of himself and the members of his command.

Summary. A naval officer, age 48, suffered his first bout of paroxysmal auricular fibrillation following an operation for a gangrenous appendix. Subsequently he experienced practically daily attacks. During a call for general quarters on a battleship he collapsed. While the heart was irregular, the standing position accentuated the palpitation. Quinidine was essential to maintain sinus rhythm. Premature ventricular systoles were often observed during the auricular fibrillation. During regular rhythm the heart was slow and there was no evidence of organic cardiovascular disease.

Case 4. R. E. G. (C.M.1/c). This patient was a 39-year-old "Seabee." He had scarlet fever at the age of 2 but there was no history of rheumatic fever, chorea or diphtheria. About 8 years before he had suffered from "heart trouble" for which he was disabled about 5 months. Details of this illness were unobtainable, but apparently he had an episode similar to that for which he was admitted to this hospital. During the ensuing 8 years, he had 5 similar attacks, which lasted from 2 days to 1 month. Three weeks before admission to this hospital, he noticed the sudden onset of a sensation of "gaseous distension" and pressure on the heart followed by palpitation. He felt weak and tired since the onset of these symptoms but experienced no pain or dyspnea.

Physical examination at the time of admission revealed no abnormalities except the auricular fibrillation. The heart was not enlarged. No murmurs were heard and no accentuations were present. Apical rate was 84 and absolutely irregular; the pulse rate was 80. A pulse deficit of 4 was therefore present. Numerous moist râles were heard at the lung bases, but a Roentgen ray film of the chest taken the same day showed no

alterations of the lung fields. There was no edema of the extremities.

An electrocardiogram taken at this time indicated the existence of typical auricular fibrillation with ventricular rate varying between 60 to 80 a minute. However, there was no evidence of myocardial or valvular involvement. The QRS complexes and T-waves of the electrocardiograms were quite normal. The patient stated that his attacks seemed to follow exertion and possibly indigestion. One week later, normal sinus rhythm supervened, without the aid of medication. Examination of the heart at this time indicated normal size and contour; in fact the heart appeared small. No murmurs or significant accentuations of the heart sounds were noted. The blood pressure was 112/68. Cardiovascular studies were negative. The heart was small, with normal contour and good pulsation. The basal metabolic rate was plus 5. Blood counts, Kahn test, and sedimentation test were negative. The patient never smoked. He was evacuated to the mainland and at the time of discharge the cardiac function and rhythm were perfectly normal.

Summary. A 39 year old man developed attacks of paroxysmal auricular fibrillation, related to "gaseous distention" and exertion. He felt weak and tired. On admission he was probably in congestive heart failure for a very short time. Subsequently no evidence of organic cardiovascular disease was obtained. The ventricular rate was slow even during the arrhythmia.

CASE 5. B. H. McM., male, age 35, weight 145, height 5′ 9″. This patient was aware of palpitation for the past 15 years. At the inception of these attacks the palpitation was mild, but as the years passed these sensations became more pronounced and more frequent. At times he noticed only isolated and short sensations of irregular beating. At other times, the unsystematic heart behavior became established for from several minutes to several hours. The duration of the longest attack was 4 hours. The paroxysms began and ended abruptly and were apt to occur after meals either in the morning or the afternoon. The patient associated a feeling of distension with the onset of these attacks. He did not know how the irregularity was affected by exercise because he remained very quiet while the palpitation was present. When he was not having symptoms he could exercise vigorously but he noticed that the attacks were apt to come on afterwards, while he was resting. Relief of gastric distension was obtained by taking Coca-Cola or Alka-Seltzer, which often caused the palpitation to disappear.

The past history was entirely irrelevant. He had been a refrigeration engineer before being drafted into the service 4 months previously. Since being in the service his duties consisted solely of acting as master-at-arms of the barracks. This did not entail any heavy physical or mental strain, yet the patient felt that he had been more nervous since being in the service than at any other period of his life. The patient used to be a heavy drinker but he had been very abstemious during recent years. He smoked 20 to 40 cigarettes and drank 2 to 4 cups of coffee per day.

With the exception of evidences of marked emotional instability, the physical examination was negative. When the rhythm was regular the rate was about 80 per minute; the blood pressure was 122/66. Cardiac size and contour were normal. No murmurs or abnormal sounds were present.

A typical attack occurred about 1 hour after breakfast on the day of the first examination. At this time the rhythm was totally irregular. The apical rate was about 150 beats per minute with a radial pulse deficit of about 10 to 15. The bout persisted about 15 minutes, after which the heart became regular with an occasional premature systole. The patient had been having a similar attack practically every morning of a 2-week period prior to his admission to the hospital. Electrocardiograms taken during a typical attack revealed auricular fibrillation. There was no evidence of myocardial damage. During periods when normal rhythm ensued the electrocardiograms were normal. Laboratory studies including Kahn test, urinalysis, blood count, sedimentation rate, and basal metabolism were negative.

Summary. A 35 year old refrigeration engineer with a 15-year history of frequent episodes of rapid irregular heart action. During the attacks the patient had to remain absolutely quiet. He was emotionally unstable, very nervous, smoked

20 to 40 cigarettes a day and drank 4 cups of coffee.

Comment. The auricular fibrillation was functional in origin in all our patients; no evidence of organic heart disease was found. There was no history of rheumatic fever. No hyperthyroidism was present. Physical examination, fluoroscopy, Roentgen ray films, electrocardiogram before and after exercise[6] ("2-step"), basal metabolic rate, blood counts, urine examination and sedimentation rates all were normal. The hearts were not enlarged, there were no murmurs, no accentuations, no increased arterial tension.

Paroxysmal auricular fibrillation, functional in origin, is fairly common. It is frequently encountered in the Navy. The first 4 patients were seen in consultation within a period of but 1 week. The chief presenting symptom in our patients was "palpitation" or "pounding" of the heart. We suspect that many cases in which the diagnosis of paroxysmal auricular tachycardia is made in the history, are actually instances of paroxysmal auricular fibrillation. The patient rarely observes that his heart is irregular. Only after interrogation did 1 patient of our 5 recollect that it was irregular during the "spells" of palpitation. Paroxysmal tachycardia was the admission diagnosis of 2 of our patients. As a matter of fact, leading questions were also necessary to establish the fact that the heart rate had been rapid.

We visualize this functional type of paroxysmal auricular fibrillation very much as one does premature systoles in patients with no obvious cardiovascular disease. Ventricular premature systoles were noted in all our patients, particularly in the transition periods between the regular sinus rhythm and the irregularity or the converse. Quinidine sulphate was necessary in 2 of our patients to reëstablish regular sinus rhythm and in 2 to maintain it. The same therapy is often necessary in patients who have frequent premature contractions.

The comparison between premature systoles and paroxysmal auricular fibrillation can be carried further. Premature beats are more apt to be observed in slow hearts. Every one of our patients but the fifth had a relatively slow heart rate of about 60 beats per minute when the normal sinus rhythm was present. The cause of premature contractions is obscure and classified often as toxic, infectious and neurogenic; and so is the arrhythmia under discussion. Tobacco, coffee, liquor are common causes of both. Premature systoles alone are regarded as harmless and, if not too numerous, are no cause for rating up by life insurance companies. In the same vein, a writer[10] has stated that the mortality in our type patient, that is, one with functional paroxysmal fibrillation, is no higher than in the general population.

Probably the psychogenic or nervous factor is the most potent one in the production of paroxysmal auricular fibrillation. The war has placed military personnel under unusual stress and strain and the susceptible person develops the cardiac arrhythmia through a neurogenic mechanism. Stimulation of the vagus is thought by many authors[1,4,7,11] to be a factor in the production of paroxysmal fibrillation. Nahum and Hoff,[7] and Iglauer, Davis and Altschule,[4] as a result of experimental observations on animals, established a relationship between vagus stimulation and auricular fibrillation. Altschule[1] found vagal activity a factor in auricular fibrillation in rheumatic cardiac patients. Wolff[11] observed this functional irregularity in brothers who possessed a slow heart even after exercise.

Auricular fibrillation, paroxysmal or otherwise, due to organic heart disease, has not been observed once during many months at this overseas activity. The causes of this are usually advanced coronary, hypertensive, rheumatic, and hyperthyroid heart disease. It is reasonable to assume that personnel with such heart disease would not pass the physical examination at the time of induction into het Navy or an examination before overseas duty.

Patients with functional paroxysmal

auricular fibrillation should be discharged from the Navy or at least placed on limited duty ashore. We say this in spite of the fact that authors of numerous articles on this subject almost universally report the harmless nature of the cardiac irregularity. Willius[10] states that the death rate in his patients was similar to that in the general population. We believe that his series was too small and the period of his follow-up too short. Parkinson and Campbell[9] and Orgain, Wolff and White[8] also emphasized the good prognosis of patients with this functional paroxysmal auricular fibrillation.

A review of the 5 cases we are reporting is enough to prove that the patients were not fit for general duty in the Navy. The Chaplain had to abandon his duties on the battlefield of Saipan. The aviation machinist's mate could not perform strenuous exertion during the arrhythmia. The Lt. Comdr. collapsed during a call to general quarters on a battleship. The 39-year-old Seabee was in congestive failure during the episode which brought him to the hospital. The last patient had to remain "very quiet" during the anomalous action of his heart. Furthermore, he had become emotionally unstable.

In civilian practice, attacks of this functional arrhythmia often prove severe enough to produce definite temporary disability. Pulse deficits are present, dizziness, faintness and occasional unconsciousness occur; often the patient is quite alarmed. Comeau[2] reported syncopal attacks associated with paroxysmal auricular rhythm and the establishment of auricular fibrillation. In other words, the patient is not fit for heavy work or for employment in which he is under mental strain. Thus the senior author has had the opportunity of observing this condition in engineers of "crack" trains of a large railroad company. These engineers had to be removed from their responsible jobs.

Treatment. The treatment of paroxysmal auricular fibrillation is first, removal of the cause. In Case 3, chewing tobacco and drinking coffee may have been factors. In the last patient, the overseas duty played havoc with the patient's mental equilibrium. From the moment he learned that he was to be evacuated to the States, he became a pleasant, natural person.

Excessive exertion, mental and physical fatigue, lack of sleep, overindulgence in tobacco and coffee, gastric distension, and so forth, must be avoided. From previous experiences the victim will probably know what means are helpful in stopping an attack. Position of the body is important. Bending the trunk well forward with the head low,[5] lying on the back and occasionally lying on the abdomen bring relief.

Complete rest is sought by the patient, for he has found that exertion prolongs the attack and aggravates its severity. Occasionally the converse is true; some physical movement like stretching the body upward is helpful. The patient should be reassured, for the cardiac irregularity may alarm him. Sedatives are often helpful.

Carotid sinus and ocular pressure should be tried, one side at a time, since reflexly this may cause cessation of the paroxysm.

Frequently the duration of the anomalous rhythm is so short that no treatment is necessary. However, to establish and maintain a regular sinus rhythm, quinidine sulphate and digitalis may be required. Given together they are synergistic and quite effective but require more care when dispensed this way. Usually quinidine alone suffices. Quinidine sulphate is first administered 3 times the first day in 0.2 gm. (3 gr.) doses. If the arrhythmia persists, the drug is given q4h, then q3h, even during the night, for quinidine is excreted in a few hours. To obviate awakening the patient during the night, a double quantity is dispensed at midnight and the 3 A.M. or 4 A.M. dose is omitted. The next step, if the cardiac irregularity is still uncontrolled, is to double the quantity of quinidine, that is, 0.4 gm. (6 gr.) instead of 0.2 gm. (3 gr.) in each dose.

If heart failure is present digitalis should be administered immediately and the patient digitalized within 24 hours. The

ventricular rate should be slowed to about 50 to 75 beats per minute. A heart beating at this rate is efficient even if irregular. Quinidine is then prescribed as previously outlined.

When the last patient was to be treated, quinidine was no longer available and so potassium acetate was used. This was administered in 1- or 2-gr. doses 3 times a day and promptly produced a regular sinus rhythm. However, when the drug was discontinued the auricles began to fibrillate again within 24 hours.

Paredrine hydrobromide[3] has been recommended for paroxysmal auricular fibrillation because of its sympatheticomimetic effect.

Summary. Paroxysmal auricular fibrillation, unassociated with organic heart disease, is not infrequent in the Navy. Emotional and mental strain, associated with the war, and excessive use of tobacco and coffee, are the probable factors in its production.

Palpitation of the heart is the chief presenting symptom. The "rapid" heart is occasionally noticed but the irregular heart action is rarely observed by the patient.

An erroneous diagnosis of paroxysmal tachycardia is frequently made by the physician. An electrocardiogram taken during an attack will reveal a typical auricular fibrillation.

Paroxsymal auricular fibrillation is observed in patients whose normal rate is slow and who often have ventricular premature beats. The etiology of both these arrhythmias, when functional in origin, is probably neurogenic. Quinidine sulphate is the treatment for the two.

Digitalis should be administered if heart failure is present or impending. Potassium acetate may be successful when quinidine sulphate is not available. Before recourse is made to any drug, other methods for stopping an attack should be essayed. Reassurance, complete rest, certain positions of the body, carotid sinus or ocular pressure are first to be tried. The paroxysms may be prevented by avoiding excessive exertion, mental and physical fatigue, lack of sleep, excessive use of tobacco and coffee, and gastric disturbances.

Patients with this functional arrhythmia should be discharged from the Navy or retained for limited duty ashore only. They cannot carry on their regular assignments and are a danger to themselves and their comrades. Even in civilian practice there are times when this irregularity of the heart produces at least temporary disability, and those subject to it cannot perform heavy work or work associated with unusual mental strain.

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THE EFFECT OF SODIUM SALICYLATE ON THE SEDIMENTATION RATE OF ERYTHROCYTES IN VIVO

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The action of sodium salicylate on the sedimentation rate of erythrocytes (Corrected Sedimentation Rate—C.S.R.) in rheumatic fever is well known[4] and used to measure the therapeutic effectiveness of this drug.[2] It is also known that adequate amounts of sodium salicylate will slow down the accelerated C.S.R. *in vitro* under certain conditions.[1,7] It is therefore of interest to establish whether this effect of salicylate, *in vivo*, is specific to rheumatic fever or whether it also occurs in conditions where the accelerated C.S.R. is not due to the rheumatic reaction.

This report deals with 3 patients who had widespread carcinomatosis with elevated sedimentation rates, in whom the administration of sodium salicylate by slowing the sedimentation rate demonstrated that this effect of the drug is not limited to rheumatic fever.

Methods. Five patients were chosen because they had elevated C.S.R.'s due to metastases of carcinomas of various organs. Intractable pain due to bone metastases was the indication for salicylate therapy. Two of the patients died during or shortly after the administration of sodium salicylate and the data obtained from them are not used here for this reason, although they are comparable to those observed in the remaining 3 patients who survived the administration of salicylate by 1 week or longer.

Patient 1 was a white female of 65, operated on in 1940 for carcinoma of the breast. During 1942–1943, pains in her legs developed due to metastases in the 5th and 6th dorsal vertebræ. In 1944, metastases were noted in the right lower lobe of the lung and the patient was admitted to the hospital because of paralysis of both legs and pain. Salicylate therapy was instituted on Oct. 24, 1944, and the experiment was discontinued on Nov. 4, 1944. The patient developed pulmonary congestion which responded to digitalization and she died on November 18 of a left hemiplegia due to a cerebral metastasis found at autopsy.

Patient 2 was a white male of 80 years appearing about 70 years old, and admitted for intractable pain in both legs and swollen inguinal lymph nodes on the left. He had a chronic silico-tuberculosis which had been diagnosed 10 years earlier. Roentgen rays of the pelvis showed round scattered areas of decreased opacity in the left ilium. The prostate was enlarged but smooth. The diagnosis of metastatic carcinoma to bone and lymph nodes was made, the primary site remained unknown. The patient received salicylate therapy on March 19, 1945, and was followed until March 29. He remained in the hospital until May 14, when he died of an intercurrent infection. No postmortem examination could be done.

Patient 3 was a white male of 69 who had been operated on for carcinoma of the prostate in 1941. Two years later he noticed dorso-lumbar pain which became worse until his admission to the hospital on May 1, 1945, when pain due to vertebral metastases prevented use of his legs. Some rounded well-defined shadows were seen in both lung fields. This patient was given salicylate therapy from May 7 to June 11, 1945. He is at present about to undergo orchidectomy.

These patients were given sodium salicylate by mouth and by vein in the following amounts. (Sodium salicylate was given intravenously in a 1% solution of the salt contained in a 5% dextrose solution in distilled water.) Patient 1 received 10 gm. intravenously on the 1st day, 5 gm. intravenously on the 2nd day and 1.5 gm. by mouth every 4 hours, day and night on the 3rd and 4th day. Patient 2 received 4 gm. intravenously daily for 3 days and 6 gm. intravenously daily for 2 days. Patient 3 was given 4 gm. by mouth daily for 4 days, then 8 gm. by mouth for 2 days and thereafter 10 gm. by

mouth every day with, in addition, 6, 3.6 and 2.4 gm. intravenously for 3 consecutive days. The oral doses were then discontinued and 8 gm. given intravenously every day for 4 days. For 2 more days the patient received 4 gm. daily, intravenously.

The following determinations were made before therapy was started and daily thereafter: (1) C.S.R. according to Rourke-Ernstene.[9] (2) Plasma fibrinogen levels according to Cullen and Van Slyke.[3] (3) Plasma salicylate levels according to Co-

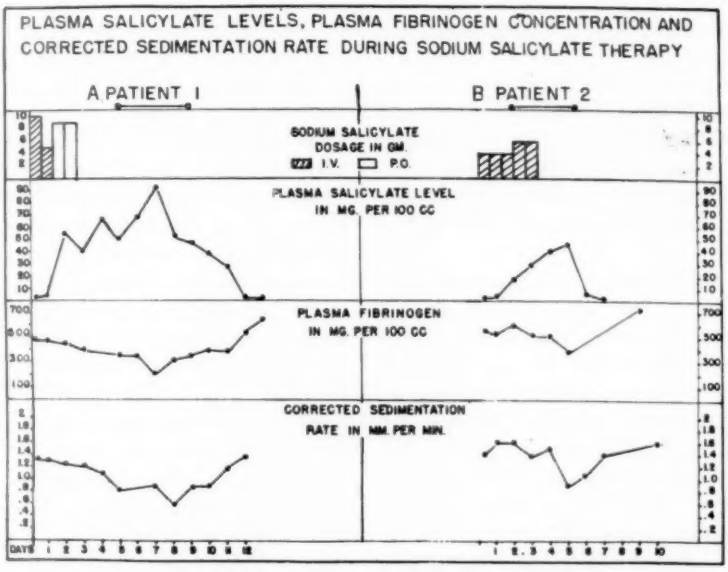
PLASMA SALICYLATE LEVELS, PLASMA FIBRINOGEN CONCENTRATION AND CORRECTED SEDIMENTATION RATE DURING SODIUM SALICYLATE THERAPY
A PATIENT 1
B PATIENT 2
SODIUM SALICYLATE DOSAGE IN GM.
I.V.
P.O.
PLASMA SALICYLATE LEVEL IN MG. PER 100 CC
PLASMA FIBRINOGEN IN MG. PER 100 CC
CORRECTED SEDIMENTATION RATE IN MM. PER MIN.
DAYS

CHART 1.—Results obtained in Patients 1 and 2.

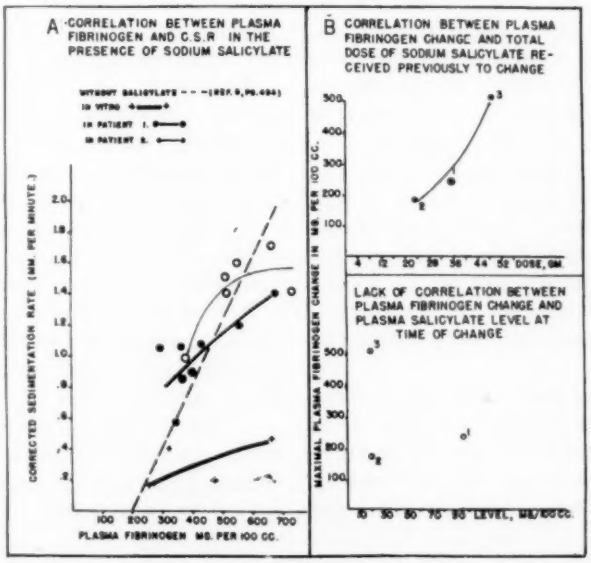
A CORRELATION BETWEEN PLASMA FIBRINOGEN AND C.S.R IN THE PRESENCE OF SODIUM SALICYLATE
CORRECTED SEDIMENTATION RATE (MM. PER MINUTE)
PLASMA FIBRINOGEN MG. PER 100 CC.
B CORRELATION BETWEEN PLASMA FIBRINOGEN CHANGE AND TOTAL DOSE OF SODIUM SALICYLATE RECEIVED PREVIOUSLY TO CHANGE
MAXIMAL PLASMA FIBRINOGEN CHANGE IN MG. PER 100 CC.
DOSE, GM.
LACK OF CORRELATION BETWEEN PLASMA FIBRINOGEN CHANGE AND PLASMA SALICYLATE LEVEL AT TIME OF CHANGE
LEVEL, MG/100 CC.

CHART 2.—*A*, Correlation between plasma fibrinogen and CSR. *B*, (upper) Correlation between plasma fibrinogen change and total dose of salicylate received previous to change. B. (lower) Lack of correlation between plasma fibrinogen change and plasma salicylate level.

burn.[2] (4) Prothrombin time determinations according to Quick.[10]

Findings regarding the prothrombin concentration will be reported later.

Results. (Chart 1, *A* and *B*.) In Patients 1 and 2 the results were comparable. After a latent period of 3 to 4 days while the plasma salicylate levels ascended, the plasma fibrinogen level and the C.S.R. did not materially change except for a slight increase of both at the beginning in Case 2. About 24 to 48 hours after the highest level of plasma salicylate concentration had been reached, the plasma fibrinogen level and the C.S.R. dropped toward normal levels.

In the third patient the plasma fibrinogen fell from 700 mg./100 ml. to 240 mg./100 ml. within the first 11 days with salicylate levels below 30 mg./100 ml. While this was comparable to the response observed in the 2 previous patients the C.S.R. did not follow the decrease of the plasma fibrinogen in the expected manner. It was then found that this patient's plasma globulin was 3.2 gm. which is enough to markedly accelerate the C.S.R. at plasma fibrinogen concentrations from normal to approximately 500 mg./100 ml. (Ham and Curtis.[5]) Later this patient developed sacral decubitus and in spite of salicylate levels as high as 60 mg./100 ml. the plasma fibrinogen level remained at 389 to 545 mg./100 ml. thereafter.

Comments. (Chart 2, *A* and *B*.) In these debilitated patients whose C.S.R. was elevated because of metastases of carcinomas, therapeutic doses of sodium salicylate clearly caused a fall of the plasma fibrinogen concentration (in 1 case following a slight increase) and a slowing of the C.S.R.

This slowing of the C.S.R. is due to the depression of the plasma fibrinogen level as sodium salicylate did not significantly modify the usual linear relation between the plasma fibrinogen concentration and the sedimentation rate. Chart 2B shows that this relation is influenced much less than *in vitro*.

As the addition of sodium salicylate to plasma *in vitro* did not produce a fall of the fibrinogen concentration,[7] it is unlikely that the mere presence of sodium salicylate in the blood would cause the observed plasma fibrinogen reduction. There is no actual relation between the magnitude of the change in plasma fibrinogen concentration in the patient and the plasma salicylate level at that time. However the change in plasma fibrinogen concentration is related to the total dose of salicylate received by the patient previously to the maximal change (Chart 2, B).

The plasma fibrinogen changes observed in these 3 patients are comparable to the findings of Irish and Jaques[8] in dogs after administration of dicumarin. They also recall the observations of Ham and Curtis[6] in patients with liver disease. The findings suggest that in the conditions of this experiment sodium salicylate is a hepatotoxic agent, or interferes with some other factor of fibrinogen regulation. It is questionable whether the C.S.R. is a reliable criterion for evaluation of the therapeutic effects of salicylates in rheumatic fever, as its depression by salicylate is unspecific.

Summary. 1. Therapeutic doses of sodium salicylate reduced the plasma fibrinogen concentration and the sedimentation rate of erythrocytes in patients with generalized carcinomatosis.

2. This effect can be explained by the depression of the plasma fibrinogen concentration by sodium salicylate.

3. The plasma fibrinogen is proportionate to the total dose of salicylate received by the patient and independent of the blood salicylate level.

4. The reported reduction of plasma fibrinogen following the administration of sodium salicylate is comparable to that caused by liver toxins.

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PROGRESS
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UNDER THE CHARGE OF
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SEROLOGIC TYPES OF BACTERIA AS AN EPIDEMIOLOGIC PRINCIPLE

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EVER since the discovery of the casual relationship of microbes to disease, the methodology of the microbiologist has stressed attempts to differentiate the "pathogen" from an infinite variety of bacterial life. In the early bacteriologic literature, the constant association of a particular microörganism with a pathologic condition was sufficient for the assumption of an etiologic relationship. The widespread dissemination of microscopic life was but little appreciated. When Loeffler found his diphtheria bacillus in the throat of a man without even a suggestion of diphtheria, it almost upset his theory. Even the postulates devised by Koch did not leave room for the healthy carrier of disease producing microörganisms. And yet the school of thought which denied the significance of the newly-discovered microörganisms opposed the teachings of Pasteur and Semmelweiss on the etiology of puerperal sepsis on the grounds that the same microbes could be found in persons not ill with the disease.

The past three or four decades—often referred to as the "Golden Age of Bacteriology" have brought an endless succession of tests and techniques, all designed to differentiate the pathogens and elucidate the meaning of its presence in the carrier as well as the disease process. The increasing knowledge of the natural history and biology of bacteria, and the concurrent development and improvement of techniques in related fields such as biochemistry and immunology have resulted in the modern serologic techniques available to the bacteriologist and epidemiologist for the classification of bacteria. The antigenic analysis of bacteria by these refined techniques has increased immeasurably our knowledge of the biology of bacteria and has provided a stability and reproducibility in systematic classification which was conspicuously lacking in earlier systems based entirely on morphologic or biochemical characteristics. However, the serologic separation of the various bacterial species into groups, types and subtypes has, on the whole, contributed remarkably little towards its original purpose—the recognition of the pathogen; or, for that matter, towards the basic

mechanics and processes involved in infection. Indeed, present-day knowledge of "virulence" in most cases is but little advanced from that of Weigert,[113] who as long ago as 1875 asked whether the "something" that renders a microörganism virulent is a vital bacterial product or merely "something" attached to the microörganism.

Systematic Serologic Classification. Landsteiner,[66] Wells,[115] and Zinsser, *et al.*[121] as well as several others have called attention to the extreme complexity of cellular and protoplasmic antigens as compared with isolated and purified antigens. Species specificity usually can be demonstrated for intact erythrocytes with high-titer species-specific antisera. Nuttall[83] showed that serologic results in general confirm zoölogic classification. However, no one knows exactly what constitutes a bacterial "species." While the available evidence suggests that each so-called bacterial "species" possesses a species-specific antigen, the existence of such a fraction cannot always be demonstrated by serologic methods employing unaltered antigens. Indeed, the results of agglutination and absorption tests suggests that most bacterial "species" are not represented in the zoölogic plan by a species-specific antigen, but, rather, by two or more antigenic types or sub-types. It is only by the use of special techniques that a species-specific antigen can be demonstrated among many of the bacteria.

In general, most bacterial species can be placed into one of three broad groups by serologic methods. The following is a partial list of the commoner microörganisms falling into each of these three groups:[104]

I. Species Identified With a High-titer, Species-specific Antiserum.

1. *E. typhosus*
2. *M. tuberculosis*
3. *P. pestis*
4. *H. pertussis*
5. *Br. abortus*
6. *T. pallidum*
7. *S. aureus*
8. Certain Clostridia

II. Species Represented by a Number of Distinct Antigenic Types, Some Sub-types and Perhaps a Heterogeneous Group.

1. *S. hemolyticus*
2. *N. gonorrhœa*
3. *N. meningitidis*
4. *Shigella*
5. *Salmonella*
6. *Friedländer's bacilli*
7. *Pneumococci*
8. Certain Clostridia

III. Species of Extreme Antigenic Heterogeneity, Immune Sera of Little Value in Species Identification.

1. *Escherichia coli*
2. *Pseudomonas æruginosus*
3. *C. diphtheriæ*
4. Aerobic spore bearers
5. *S. viridans*
6. *B. proteus*
7. Certain Clostridia

Perhaps the most thoroughly studied bacterial species, with respect to serologic classification, are the pneumococci, the meningococci, the influenza group, the enteric group and the streptococci. Among these four species, well over 200 separate serologic types have been described. In the pneumococci, meningococci, clostridia and influenza group (especially the latter), serologic classification has (and still has in certain instances, regardless of drug and antibiotic therapy) a therapeutic usefulness, irrespective of whether or not the type-specific antigens are related to the ability of the microörganism to produce disease. On the other hand, there is as yet no therapeutic reason for serologic type differentiation among the enteric group or the streptococci. The pneumococci, the meningococci, the enteric group and the streptococci only will be considered in detail here, since the type classification of these microörganisms has been most extensively applied to epidemiologic studies. Of the remaining groups, two deserve mention, although type differentiation is employed almost exclusively for therapeutic reasons.

The Clostridia. The Clostridia associated with gas gangrene are unusual in that toxigenic type can be correlated with serologic type.[104] Whether or not the polysaccharide determining serologic type is a part of the toxin molecule, lending

type specificity to the various toxins of the strains in this genus is not yet known. In any case, the clinical disease induced by the various toxigenic types is essentially similar, differing only quantitatively.

Two other types of Clostridia are of interest in that each presents a similarity or contrast to the gas gangrene group. *Clostridium tetani* has been separated into at least six serologic types by agglutination tests. However, all agglutination types produce an antigenically identical toxin. On the other hand, *Cl. botulinum* is another exception to the general rule that different strains of the same microörganism produce the same toxin. At least five types of *Cl. botulinum* exist, each of which produces a toxin antigenically distinct from that produced by other types.

The studies of Meyer, *et al.*[76] on differences in the geographic distribution of the various toxigenic types of *Cl. botulinum* suggests an epidemiologic reason for the observation that toxigenic type causing the majority of cases of botulism varies with geographic location.

The Influenza Group. Although the serologic classification of *Hæmolytica influenzæ* is of foremost importance in the therapy of influenzal meningitis, few epidemiologic studies have been done on the distribution of the various types. It has been observed that the carrier rate for untyped *H. influenzæ* does not appear to show the seasonal fluctuations observed in the carrier rates for other microörganisms.[8]

Any one of six serologic types of *H. influenzæ* occasionally produces influenzal meningitis. However, Type B is the predominant type associated with disease. Silverthorne and Paterson[106] have shown that carriers of *H. influenzæ* Type B are rare, usually being found only in the immediate vicinity of cases, and in many instances not even under these circumstances.

The Pneumococci. The epidemiology as well as the clinical and pathologic aspects of pneumococcal infection have been reviewed by Heffron,[53] while the varied aspects of the biology of the pneumococcus have been compiled and catalogued in an extensive work by White.[119] The discussion of the pneumococci here is intended only to point out some of the epidemiologic similarities and contrasts between the relationship of the serologic types of pneumococci to pneumococcal infections and the serologic types of other microörganisms to the disease processes they induce.

The pneumococcus is perhaps second only to the streptococcus with respect to the multiplicity of ills it can inflict on mankind—lobar pneumonia, bronchopneumonia, sinusitis, otitis, mastoiditis, meningitis—to mention only a few. To date, well over sixty serologic types are recognized, and there seems to be no specific correlation between serologic type and clinical diagnosis, although certain types tend to predominate as the cause of some pneumococcal infections. For example, there seems to be a difference between the serologic types predominating in lobar and bronchopneumonia. Heffron[53] indicates that in general, Types I, II and III are the predominant causes of lobar pneumonia, and states that the distribution of types in bronchopneumonia approximates the distribution of the various types in the buccal cavities of normal persons. Heffron presents a table based on data from East Africa, Germany, Great Britain, India and the United States in which Type I appears in 6.3% of the cases of bronchopneumonia, Type II in 4.3%, Type III in 14% and the remaining types (Group IV) in 75.3% of all cases reported. The total percentage of cases accounted for by Types I and II combined (10.6%) is in sharp contrast to the total percentage of cases of lobar pneumonia (53.4%) caused by Types I and II (Table 1).

These data give only a composite picture of the world at large. There are, of course, seasonal and local variations in the predominance of the various types in pneumonia at a given time.[119] Family and

institutional outbreaks[95,109] have been caused by various types at different times and in different places; such outbreaks usually being due to a single serologic type.

PNEUMOCOCCUS CARRIER RATES. It has been claimed that the frequency of pneumococcus carriers increases with the advent of cold, unsettled weather.[15] Such observations suggest the traditional interpretation, *viz.*, that an increased carrier rate as a result of "crowding" precedes the appearance of pneumococcus pneumonia.

Pneumococcus carriers may be transient, chronic or sporadic. In any case, the pneumococcus types predominating in lobar pneumonia are usually rare among carriers other than those convalescing from lobar pneumonia or in the immediate vicinity where cases are occurring. Gundel and Schwarz[48] for example, found that types other than Types I, II and III accounted for well over 60% of pneumococcus carriers, Type I was present in only 0.8%, Type II in 0.4% and Type III in 6.7% of the carriers studied. Rosenau, Felton and Atwater[89] isolated Type I pneumococci four times as frequently, Type III twice as frequently from persons in contact with cases of lobar pneumonia as in those with no immediate contact with cases. Curiously enough, there appeared to be little difference in the incidence of Type II among the contacts and non-contacts, while pneumococci of the remaining types were isolated from 83.5% of the contacts as compared with 69.3% of the non-contacts. On the other hand, as already mentioned, the frequency of types found in bronchopneumonia is similar to the distribution of pneumococcus types among carriers.

It would seem, then, that except under circumstances such as an institutional or family outbreak of pneumonia, a study of the carrier rates by serologic type can add little to the knowledge of the epidemiology of pneumonia. Even the presence of Types I, II and III in carriers does not necessarily predict the occurrence of cases since there is no knowledge as yet of the factors predisposing to pneumonia on the one hand, and on the other, of why some types or certain strains of the same type gain predominance over other types which may be present in a population group. The similarity of the distribution of various types among carriers and in cases of bronchopneumonia suggests that bronchopneumonia is a chance phenomenon, perhaps dependent upon changes in the autarceology of the host rather than differences in the infecting parasites.

RELATIONSHIP OF TYPE TO DISEASE. Although the ability to produce pneumonia or other pneumococcal infections is not dependent upon serologic type *per se*, there are striking differences in the mortality rates resulting from pneumonia due to different serologic types. Heffron[53] cites mortality rates of 25%, 41% and 45 to 60% respectively for Types I, II and III. The data on mortality for the remaining types are scattered, but those available suggest a mortality of around 30% in untreated (serum) cases. "Virulence" is a relative term and is dependent upon a number of factors, hence there is yet no adequate explanation as to why the mortality of Type III cases should be so excessively high. Differences in "invasiveness" probably is not the entire explanation, since pneumococcemia frequently occurs in pneumonia patients and usually presents a grave prognosis regardless of serologic type.[6] The serologic classification of the pneumococci, with the consequent studies of the capsular polysaccharides and their relationship to type-specific immunity has added little to our knowledge of the fundamental mechanics of pneumococcus infection. Despite the success of specific therapy based on serologic type, the reasons for the differences in disease producing capacity of various types, and of individual strains of the same type have not yet been explained. Whether the capsular polysaccharides are toxic in

themselves or combine with substances in the body to form toxic products, or whether the capsular polysaccharide lends antigenic specificity to a conjugated protein common to all types is not yet known. From an epidemiologic viewpoint, the ability of all types to produce disease suggests a common denominator, either host variations, or the long-sought but still elusive "pneumococcus toxin."

since so far as is known, immunization with standard typhoid vaccine protects against all bacteriophage types. Further study may reveal differences in the geographic distribution of various types in typhoid fever, probably correlating with the distribution of carriers of the various bacteriophage types.

SHIGELLA DYSENTERIÆ. The genus Shigella today is represented by twenty

TABLE 1.—PERCENTAGE DISTRIBUTION OF SEROLOGIC TYPES OF PNEUMOCOCCI IN LOBAR PNEUMONIA (14,869 CASES)

Location	Percentage distribution			
	Type I	Type II	Type III	Others
North America	31.5	19.2	11.8	37.5
Europe	38.7	28.3	7.0	26.0
Africa	19.1	12.2	2.2	66.5
Far East and Australia	41.0	15.4	6.6	37.0
Average	32.8	20.6	10.8	35.8

THE ENTERIC GROUP. The studies of Craige[20,21] on the bacteriophage typing of *Eberthella typhi*, while not strictly a serologic method, deserve mention because of their epidemiologic significance. On the basis of sensitivity to various bacteriophages, ten types and several sub-types of *E. typhi* can be differentiated. The present-day epidemiology of typhoid fever being primarily a matter of carrier-contact infection, typhoid typing has increased tremendously the value of the typhoid carrier register to the epidemiologist investigating an outbreak. If the type of organism carried by the known typhoid carriers in the vicinity is known, and the cases occurring in the outbreak are typed, it is a simple matter to incriminate or exclude a carrier of responsibility.[38] Furthermore, single source outbreaks are usually of a single type, while multiple type outbreaks usually indicate water or sewage as the source.[20,38] As with other microorganisms, the recognition of types has become of some use to the epidemiologist although type *per se* is not correlated with the ability to produce typhoid fever. Indeed, among the typhoid bacilli, at least, it is clearly evident that "typeness" is incidental to the ability to induce disease, species of medical importance, which are fairly well differentiated by biochemical as well as serologic methods.[11] Any one of these serologic types can produce clinical dysentery in man, although there are variations in the frequency with which the various types are associated with disease from time to time in different places. Epidemiologically, bacillary dysentery, or more strictly, shigellosis, since the Salmonella also may incite clinical "dysentery," is largely a matter of carrier-contact infection, either direct or food-borne. Since extensive outbreaks of the disease usually originate with a food-handler, the differentiation of these microörganisms by type is of considerable use to the epidemiologist in the search for carriers incriminated in an outbreak. However, type differentiation is of no value either in the recognition of dangerous carriers among those who give no history of having had the disease or in therapy, since all types produce an essentially similar clinical syndrome. A possible exception, insofar as therapeutics is concerned, is the reported success of sulfadiazine in cases caused by *Shigella dysenteriæ*.[114]

RELATION OF TYPE TO DISEASE. Although the distribution of various Shigella

types is known to vary over large areas, data for the United States is scanty. The studies of McGinnes, *et al.*[75] Hardy, *et al.*[51] Mayfield and Gober,[74] and Wheeler[116] seem to concur in the opinion that *Sh. paradysenteriæ* (Flexner organisms) is the predominant cause of endemic dysentery in this country. On the other hand, a recent report by Nelson, *et al.*,[80] indicates that in a study done in the southeastern area of the United States, *Sh. sonnei* was isolated more frequently than all other types combined. Even in the states covered by this survey, there was a shifting of predominant type from state to state. It appears that the Shigella types most frequently isolated from sporadic cases were those types enjoying widest geographical distribution. There is insufficient data to determine whether these most recent findings indicate a shift in the prevalence of Shigella types or are the result of local conditions.

Despite our knowledge of the bacteriology of dysentery, the answer to the problem of infection by these microörganisms is still unsolved. The clinical symptoms of dysentery, on the whole very much alike, cannot be easily reconciled with the variety of types of microörganism which are so different in their biochemical and physiologic activities. For example, a Shigella usually associated with mild disease is not infrequently recovered from a severe outbreak, while those types (*Sh. dysenteriæ* or *Sh. sonnei*), usually considered as toxic and the incitants of severe epidemic dysentery, are frequently recovered from a mild, sporadic case.

Boivan, Corre and Lehoult[13] suggest that the reason for this seemingly paradoxical situation may be that the so-called group antigen, a carbohydrate-lipid complex common to various types of Shigella as well as other Gram negative microorganisms, appears to be the "enterotoxic" factor, rather than any of the as yet recognized type antigens.

The Salmonella Group. If the existence of twenty species of Shigella is difficult to reconcile with the essentially similar clinical disease each can induce, then the types existing among the Salmonella are entirely beyond any hope of reconciliation with the clinical syndrome of Salmonellosis. At the present time, there are more than 164 strains or types of microörganisms of the Salmonella group which can be differentiated by the Kauffmann-White schema.[11] About 25% of these strains have been isolated from human infection, the remainder being of various animal or avian sources.[9] Although the majority of Salmonella types are not of immediate origin, all members of the genus Salmonella must be considered as potential etiologic agents of sporadic or epidemic gastro-enteritis and enteric fever in man.

The widespread dissemination of the genus Salmonella in man and lower animals provides a variety of possible sources for accidents of environmental sanitation giving rise to an outbreak of enteric fever or Salmonellosis. One of the earliest uses to which Salmonella typing was put was the differentiation of the organism into either the "animal" or the "human" group, thus giving the epidemiologist an indication of where to look for the source of infection. However, Rubenstein, Feemster and Smith[91] point out that by the epidemiologic and bacteriologic study of contacts of sporadic cases, institutional outbreaks, etc., they were able to demonstrate case to case spread of Salmonella infections due to several serologic types. These authors also point out that type studies have proven the importance of a temporary carrier state following infection with any one of several types, as well as the importance of missed or subclinical infection in the propagation of new cases. These same authors emphasize the existence of permanent *Salmonella schottmülleri* (*Sal. paratyphi* B) carriers. Indeed, a re-examination of the epidemiology of Salmonella infections in the light of Salmonella type-classification led these authors to question the thesis that the con-

stant interchange of Salmonella between other hosts and man rather than human to human contact is the most significant factor in the transfer of Salmonella infection. In other words, as with other infections, Salmonellosis spreads through a population by means of small groups of clinical and subclinical infections, thus considerably altering the control measures to be instituted in their control.[91] Serologic type is indispensable to the epidemiologic study and control of Salmonella infection.

Relationship of Type to Disease. As already mentioned, about 25% of the recognized types of Salmonella have been isolated from human disease. Infections due to various types of Salmonella are essentially similar clinically. Rubenstein, Feemster and Smith[91] point out that even the time-honored distinction between Salmonella fever and septicemia is perhaps as unjustified as would be a distinction between typhoid fever and typhoid septicemia.

There are certain analogies between the distribution of Salmonella types and that of the pneumococci and streptococci in disease, in that although all strains can induce disease in man, a few serologic types predominate in a given locality. In Massachusetts, for instance, between the years 1937–43, 22 types of Salmonella were isolated from cases and carriers. Of 811 cases studied, 70% were produced by four serologic types. These same four types also occurred most frequently in routine stool cultures taken for some reason other than clinical illness.[30] The data on the geographic distribution of Salmonella types is still too limited to permit generalizations as to the extent or permanence of a type-pattern, but there is some evidence suggesting variations from time to time and place to place. Seligmann, Sophra and Wassermann[103] published a comparison of the distribution of Salmonella types in Massachusetts and Connecticut, 1939–1942. The distribution was much alike in each state; *Sal. typhi murium* and *Sal. schottmülleri* (*Sal. paratyphi* B) was commonest, relatively more common in Connecticut, while on the other hand, *Sal. cholerasius* was less common in Connecticut than in Massachusetts. *Sal. senftenberg*, appearing in an institutional epidemic in Massachusetts, was not encountered in Connecticut. The data cited by Seligmann, *et al.* suggest that the rarer types of Salmonella at least are often peculiar to a certain area at a given time.

From the standpoint of therapeutics, type-specific serum therapy, as with the Shigella, is unsatisfactory. There is some evidence that type differentiation may be of some value in drug therapy, since sulfanilyl-guanidine exhibits a selective action on different types of Salmonella.[12]

The Hemolytic Streptococci. The hemolytic streptococcus is perhaps the most universally important of that group of microörganisms producing disease in man. There is no other bacterial species which is capable of producing in the human host the multitude of serious lesions and the variety of clinical manifestations as has been attributed to this microorganism, ranging from inapparent infection, a transient sore throat or tonsillitis, to the acute fulminating infections in infants and young children, or the fatal toxemias and septicemias sometimes associated with puerperal fever and scarlet fever. There are few pathogens too, which can boast of the serious sequelæ of infection—such as permanent cardiac damage or acute nephritis. Its seemingly infinite importance to the economy of mankind is re-emphasized by each addition to our knowledge of its habits and biology.

The hemolytic streptococcus has occupied the attention of the medical bacteriologist in one way or another almost continuously since its discovery. During these years a tremendous volume of literature has accumulated concerning clinical manifestations, diagnostic schema, classification, etc. An excellent historic survey of the development of the classification of streptococci from the work of Schottmüller to the serologic classifications of Griffith

and Lancefield has been published recently.[31] Perhaps the most outstanding characteristic of the early work on the classification of the streptococci was the tendency of most investigators to follow the precedent of Ogston, Fehleism, and Rosenbach by assigning species names and attributing specific pathogenic abilities to the streptococci isolated from each of the infinite manifestations of streptococcal infection. For example, the terms "*Streptococcus scarlatinæ*" and "*Streptococcus epidemicus*" appeared in the literature as the specific etiologic agents of scarlet fever and septic sore throat respectively.

The theory of species specificity among the streptococci has given rise to perhaps one of the most long-standing and heated disputes in modern bacteriology. On the other hand, the epidemiologist has long been confronted by evidence that the two are closely related. Sydenham,[111] in 1676 coined the name "scarlet fever," and referred to it as a disease in name only. As long ago as 1880 the occurrence of "sore throat" was noted in the epidemiologic study of milk-borne and contact outbreaks of scarlet fever.[99] These non-scarlatinal cases (often in siblings of patients with the classical picture of scarlet fever) were considered as "scarlatina sine eruptione," or as Trousseau referred to them, "scarlatine frust." The question of species specificity was more or less settled by Okell in 1932[84] when he demonstrated that sore throat, puerperal fever, erysipelas (streptococcal), scarlet fever and so-called surgical scarlet fever are all associated with a single hemolytic streptococcus, or possibly variants of a single type. His concise views of the unitarian concept of the pathogenesis of hemolytic streptococcal infection in man —the view that different clinical pictures were the manifestations of different equilibria between the same parasite and its hosts, did not deny the existence of antigenic types, or the preponderance of a particular type (or types) in certain kinds of infection—an analogy to the preponderance of a few particular types of pneumococci in lobar pneumonia.

Griffith[47] later succeeded in establishing the existence of antigenic types among the hemolytic streptococci, thus lending bacteriologic support to the then more or less revolutionary views of Okell. Later work in this country and in England has shown that all except four of Griffith's original type strains belong to Lancefield's Group A,[65] the serologic group to which the majority of strains producing human infection belong.

Thus, the modern classification of the streptococci is based on their antigenic structure. The majority of strains can be classified into groups on the basis of a group-specific carbohydrate. Although several serologic groups contain different serologic types, the type-classification of Group A strains is perhaps the most important as well as the most complex. Group A strains are classified as to serologic type on the basis of type-specific proteins; either by Griffith's[47] slide agglutination test involving the so-called "T" antigen, or the more recent precipitation technique involving the "M" antigen.[110] Either technique has its advantages and disadvantages; the choice of methods depending largely upon the purpose for which the results are intended. Epidemiologically, it seems that in general, one technique will yield information just as useful as the other, since the value of type classification lies primarily in the recognition of a single strain in any series of cases under study.

Heterogeneity of Serologic Types in Streptococcus Infections. Concurrent with the prolonged dispute with reference to species specific etiologies for streptococcal infections, there has even persisted an undercurrent of doubt in the minds of some, as to whether or not the hemolytic streptococcus is actually related to scarlet fever. Certain discrepancies in the chain of evidence in favor of the streptococcus, and the indirectness of some of its supporting observations rather than

any validity in the claims advanced from time to time in favor of other microorganisms[16,17,72,73] have been responsible for the retention of the idea of a non-streptococcal etiology. As recently as 1912, Frost,[36] in a report describing an outbreak of septic sore throat in Baltimore stated, "Also, while the pretty constant occurrence of a rather unusual variety of streptococcus in connection with so many cases indicates most strongly that the disease was a specific streptococcus infection, there remains at least the possibility that the streptococci were of secondary importance, as in measles and scarlet fever."

The older concept of "*Strep. scarlatinæ*" not only was erroneous, but tended to over-simplify the relationship of the hemolytic streptococcus to scarlet fever, as well as the relationship of other species-specific streptococci to other streptococcal infections. The application of serologic classification to epidemiologic studies of streptococcus infection has shown that species specificity does not exist among the streptococci even in the sense that it does among the meningococci; *viz.*, the epidemiologic relationships of Type I meningococci in cerebrospinal meningitis. On the contrary, with the streptococci there appears to be no fixed relationship between serologic type *per se* and clinical diagnosis, or, for that matter, the ability to produce disease. As stated by Brown and Schaub,[14] "*Strep. scarlatinæ*" is not *the* streptococcus of scarlet fever, but is *a* streptococcus of scarlet fever.

The serologic heterogeneity of the streptococci producing scarlet fever as well as other streptococcal infections has been repeatedly demonstrated since Griffith[47] first published his studies on the type classification of "*Strep. pyogenes.*" In view of the studies reported by the Dicks,[22] Griffith's observations were not so acceptable to those who still clung to the idea of species specificity—one disease—one toxin—one microörganism. This apparent discrepancy has now been explained by the repeated observation that the common erythrogenic toxin, the so-called "N. Y. –5" toxin is produced by most Group A streptococci regardless of serologic type. From these and the observation that serologically identical strains producing an identical toxin can be isolated from non-scarlatinal infections, has evolved the modern concept of scarlet fever, namely, that the scarlatinal rash is a symptom, not a disease. As has been pointed out by Gordon[41] and others,[98] the occurrence of a scarlatinal rash is dependent upon the susceptibility of the host to erythrogenic toxin; a Dick negative subject may contract a non-scarlatinal infection from a case of scarlet fever, and transmit the infection to contacts in whom a scarlatinal rash will appear. The Dicks[22] resurrected the term "scarlatina sine eruptione" to explain the patient infected but without a rash, since even in their inoculation experiments certain subjects became ill but did not develop a rash. However, the present day concept of scarlet fever as related to streptococcal infection in general does not permit the use of such a term.

In Table 2 it may be seen that during a given period of time in a given locality, the serologic types of hemolytic streptococci producing scarlet fever occur with about the same frequency in non-scarlatinal infections. Of the 26 original Griffith types belonging to Lancefield Group A, 22 were found to produce scarlet fever in Massachusetts during the periods of time represented in Table 2. The serologic types which do not appear in this table (Types 3, 22, 23, 30) have all produced scarlet fever at other times in different places.

Distribution of Serologic Types in Scarlet Fever. A summary of the various serologic types of hemolytic streptococci isolated from scarlet fever and from non-scarlatinal infections at different times in different parts of the world shows that no single type of hemolytic streptococcus is peculiar to a particular clinical diagnosis,

and that predominant types may vary from one country to another, or from year to year in the same country or community.[33,98,118] However, two or three predominant serologic types occur as a major cause of scarlet fever in a given community (despite fluctuations in predominance).[33,42,58,118] The occurrence of a variety of types other than those predominating in the community serves to emphasize the heterogeneous etiology of scarlet fever. Moreover, the occurrence of these same serologic types in nonscarlatinal infections emphasizes the essential unity of streptococcal infection in general.[33]

Table 2.—Percentage Distribution of Various Serologic Types of Hemolytic Streptococci in Scarlet Fever as Compared With Other Streptococcal Infections

Serologic type	Massachusetts, 1938–39		Massachusetts, 1942–43	
	Scarlet fever 340 cases	Other 297 cases	Scarlet fever	Other
15	44.4	30.6	0.2	
13	29.7	12.4	0.2	
11	6.8	17.8	0.3	
1	5.6	10.8	23.9	21.2
2	5.6	2.3	36.6	30.3
12	1.5	6.4	0.9	3.0
10	1.2	0.4	0.1	
25	0.8	7.1	1.5	6.0
5	0.6	..	1.2	
8	0.6	1.0	11.0	3.0
27	0.6	6.7	0.4	3.0
29	0.6	..	0.7	3.0
6	0.3	0.7	6.3	
9	0.3	0.7		
14	0.3	..	0.3	
19	0.3	..	12.1	18.2
28	0.3	2.7		
26	..	0.4		
17	..	..	3.6	12.1
4	..	..	0.3	
18	..	..	0.3	
24	..	..	0.1	

Fluctuations in the streptococcus type pattern of scarlet fever, with the disappearance of certain types and the appearance or reappearance of certain other types, is probably the result of the loss or acquisition of the ability of the strain to produce erythrogenic toxin. Although there is no knowledge of the circumstances which favor or inhibit the production of erythrogenic toxin by a given strain, epidemiologic observations suggest that serologic types vary in toxin production from time to time and from place to place. The appearance and persistence of a predominant type pattern in a community suggest that locally and temporally certain types are the most successful rash producers.[118] The serologic types of hemolytic streptococci predominant in scarlet fever might be compared in epidemiologic behavior with the pneumococcus types predominant as causes of pneumonia.

Epidemiologic Significance. Aside from establishing the heterogeneity of the hemolytic streptococci and the essential unity of scarlet fever and other streptococcal infection (which, by the way, seems to have been Griffith's original purpose) the separation of Group A into its component serologic types has contributed little as yet to our knowledge of the fundamental mechanics of streptococcal infection. The development of type classification led to the hope that not only would "virulence" be explained, but that the epidemiology of streptococcal infection could be more clearly elucidated by relating the carrier to the case or the case to

another case in an orderly chain of epidemiologic circumstance. However, the finding that any serologic type may produce either scarlet fever or non-scarlatinal infections, together with fluctuations in the prevalence of the various types in the same population groups with reference to time and space, not only indicates that the ability to produce disease does not depend upon serologic type, but also that serologic type is of limited value in clarifying the epidemiology of endemic disease.

Although in clearly circumscribed outbreaks, one serologic type is usually the cause of disease,[25] such is not the case in the endemic form of scarlet fever as it occurs in this country today. The movement of a particular serologic type is followed with difficulty among the population in even a relatively small community. Spot maps by street address of the cases occurring over a period of 2 years in a local community failed to reveal any significant special grouping of cases by serologic type.[33] In another instance, 94 cases of scarlet fever occurred in a semi-rural community with a population of less than 7000, after an absence of 2 years. Investigation revealed that seven serologic types were involved in these few cases, the various types appearing simultaneously and showing similar fluctuations in prevalence throughout the winter and early spring months.[94]

The modern concept of scarlet fever (and other streptococcal infections) embraces both the case and the carrier as sources of the new case. Although serologic type has enabled us to detect the carriers and sort them out by type, the rôle of the carrier in streptococcal disease is as yet more or less undefined. As pointed out recently,[92] the so-called "carrier surveys" have not distinguished between the healthy or "normal" carriers and the convalescent case. Since most carrier studies have been done at the time and in the place that scarlet fever was occurring, such a distinction may have a direct bearing upon the problem.

The seasonal increase in hemolytic streptococcus carrier rates has been interpreted as a precursor of an outbreak of streptococcal disease. Schwentker, Janney and Gordon[98] noted a cyclic rhythm in the gross and Group A carrier rates, the peak being reached during the late autumn and winter months, and the lowest in the summer months. As regards season, the same cyclic phenomenon has been observed in New England,[33] and can be correlated with the seasonal incidence of streptococcal disease, either in New England or the U. S. Registration Area.[33] Such similarities in the frequency distribution of two phenomena suggest a basic underlying cause common to both. Hence, it appears impossible to distinguish between cause and effect—whether or not streptococcal disease is dependent on the carrier rate or *vice versa*. Since the same types producing disease usually are found among the carriers, the evidence for either interpretation is confusing.

In the Roumanian study[98] it was observed that some factor other than the lack of toxigenic strains must be responsible for the absence of scarlet fever since there was little difference in the streptococcal flora between communities with and without scarlet fever. Incidentally, the distribution of Dick positive individuals is not the entire answer since the distribution by various age groups in communities free of scarlet fever was much the same as in other parts of the world, and in those communities when scarlet fever occurred, the distribution of positive Dick reactions was similar to the age distribution of scarlet fever.

Schwentker, Hodes, Kingsland, Chenoweth and Peck,[97] in a study of a company of naval recruits, observed that the type pattern observed in streptococcal illness was essentially the same as that found among the carriers. Where more than one type of hemolytic streptococcus was producing disease, it was found that the number of cases produced by a given type was proportional to the carrier rate for

that type. In this instance, the streptococcus carrier rate was high—69% as compared with the usual 10% in the general population. On the other hand, a sharp increase in the cases of streptococcal illness has been observed in a population group which showed no increase in the carrier rate over the level maintained prior to the appearance of cases.[32] In a study by Hamburger, Hilles, *et al.*[50] it seemed that despite a changing population, the type pattern of cases of streptococcal illness admitted to an Army Station Hospital remained fairly constant over a period of 2 years, suggesting that the problem is not entirely a question of the accumulation of carriers in a population group. Hamburger[49] further observed that with as few as one or two streptococcus carriers present, cross infection may spread rapidly on open wards, while on the other hand, cross infection might not occur on other wards where the streptococcus carrier rate was as high as 50%.

Coburn[18] suggests that such differences in the dissemination of a particular type at different times in different places, is the result of adaptive changes by the microörganism with relation to the changing immune state of the host under the conditions of infection. This property of adaptation may be quickly lost, but so long as it exists the microörganism possesses a high degree of "communicability." Some support is found for such a theory in the observation that the incidence of streptococcal infection is directly proportional to the carrier rate for the serologic type producing the cases. However, it has been repeatedly observed that the incidence of streptococcal infection (and streptococcal carriers) follows a definite curve related to progression of season.[33] Furthermore, the seasonal fluctuation in the streptococcus carrier rate has been shown to be due to variations in the incidence of Group A strains, the non-Group A strains maintaining a more or less constant level throughout the year.[32,96] It seems, then, that if an increase in the carrier rate or in "communicability" precedes the appearance of streptococcal infection (rather than *vice versa*) the non-Group A flora of the human throat should show a seasonal variation similar to that of the Group A flora, unless one is willing to apply a hypothesized seasonal difference in "transmission" or "communicability" to a particular group of strains. In any case, regardless of the nature of the mechanisms involved, it seems apparent from the accumulated evidence that the ability to "spread" or produce clinical disease is not related to serologic type.

It is not to be inferred that the carrier plays no part in the dissemination of streptococcal disease. Coburn[18] described a series of cases directly attributable to convalescent carriers. Studies on scarlet fever in schoolrooms[93] and in households[35] reveals the importance of the convalescent carrier and the subclinical case in the dissemination of infection. The point is that even with the present day knowledge of serologic type at our disposal we can neither ascribe exact significance to the so-called "healthy carrier," nor can we sort out and segregate the "dangerous carrier" by the nature of the microörganism he harbors.

On the other hand, fundamental concepts of the epidemiology of streptococcal infection may be gained by the application of serologic classification to the study of the spread of infection in basic epidemiologically related units of population. Milk-borne outbreaks, for example, usually are due to a single serologic type of streptococcus, and much has been gained concerning differences in the clinical manifestations of streptococcal infection in different hosts exposed to the same strain of microörganism.[25,107] It has been found, too, that single type outbreaks of streptococcal disease are not uncommon in schoolrooms,[93] institutions,[61] and households,[35] when the application of serologic classification enables the epidemiologist to trace the spread of infection through the contacts of the primary cases. It is of value,

too, in the study of the effect of chemoprophylaxis on carriers and cases[92] and in the control of cross infection on open wards and in institutions, especially those devoted to the case of rheumatic fever patients.[49,61]

ANTIBACTERIAL IMMUNITY. One of the earliest attempts to immunize against streptococcal infection (scarlet fever), came soon after and followed the pattern of Jenner's work on smallpox. In a book published in 1806, Becker[10] described a method then in use. Blood from the cut skin of a scarlet fever patient was rubbed into scarifications on the arm of the person to be immunized. During an epidemic of smallpox in a small Ohio village, Hudson[56] tells of seeing a man who had been exposed to smallpox and thought he had it. Hudson vaccinated the whole family, and returning next day, found that his patient had scarlet fever and not smallpox. Believing he had accidentally found a method of immunization against scarlet fever, Hudson suggested that smallpox vaccination be done early in the course of scarlet fever, and the resulting scales be used to immunize others. Hudson actually used the scabs from the arms of 2 boys in the family he first visited to immunize about 30 children, of which 23 developed mild scarlet fever. Although the discovery of erythrogenic toxin has resulted in considerable knowledge of antitoxic immunity, the sum total of knowledge concerning antibacterial streptococcus immunity and methods of inducing it are almost as vague as Hudson's primitive immunization techniques.

In general, although the erythrogenic toxin of most serologic types is antigenically similar, the immunologic response in the patient may be monovalent, suggesting a specific response to the type of infecting organism. Thus, if the patient is reinfected during convalescence with another type of toxigenic strain, he may suffer a relapse of scarlet fever.[67] On the other hand, given sufficient time, the immunity following a single attack becomes polyvalent, resulting in more or less permanent immunity to the erythrogenic toxin. This immunity is primarily antitoxic, however, and does not necessarily protect against subsequent bacterial invasion by the same or other serologic types of streptococci.

Relatively little is known about antibacterial immunity to streptococcal infection; most of the observations and investigations in the past having been concerned with antitoxic immunity. It has been observed frequently that Dick positive individuals may harbor the identical streptococcus producing scarlet fever in other individuals at the moment without developing clinical or subclinical infection. The evidence that such immunity is of an antibacterial nature is indirect; for example, Kuttner and Krumwiede[61] observed that rheumatic children usually do not contract streptococcal pharyngitis due to the same type more than once. However, its exact nature, whether it is type or group specific, and whether it is of parasitic or autarceologic origin is unknown.

The earlier attempts to demonstrate antibacterial immunity, all generally unsatisfactory, by agglutinin, precipitin, complement fixation and opsonin tests have been reviewed by the Dicks.[22] More recently, the investigations of Loewenthal[68] and Lyons[69] suggest that an antiserum capable of protecting experimental animals must contain homologous type-specific antibodies. Platov, Dwan and Hoyt,[85] Kuttner and Lenert[62] and more recently Rothbard[90] have demonstrated feeble type-specific antibacterial activity in convalescent antisera. These recent observations tend to support the opinion that the type-specific "M" protein is associated with "virulence." There is evidence that the "M" protein is at least partially responsible for the protective effects of antibacterial sera and vaccines,[70] and that the anti-"M" antibody brings about opsonization of encapsulated streptococci. These observations might be taken to indicate that the "M" substance

is responsible for streptococcal virulence, hence antibacterial immunity should be type-specific. However, it should be remembered that this material has been obtained in equally large amounts from avirulent strains[64] and, on the other hand, may not be contained in strains of unquestioned human "virulence."[32] That it may be possible to stimulate protective antibodies with a substance not in itself related to "virulence" or the ability to produce disease is illustrated by the non-type specific immunity which can be obtained with the pneumococcus.[108]

Factors Other Than Serologic Characteristics in Relation to Virulence. The accumulated evidence on the distribution of serologic types and groups in human disease lends support to the thesis that serologic characteristics are incidental to the ability of the streptococcus to produce disease; suggesting the existence of an as yet undefined basic phenomenon or property common to most streptococci.

Kendall, Heidelberger and Dawson[59] have shown that hyaluronic acid, a polysaccharide originally described in vitreous humor, umbilical cord and synovial fluids[77] is one of the main components of the hemolytic streptococcus capsule. Seastone[102] reported experiments in which it was shown that this substance was undoubtedly related to the virulence of hemolytic streptococci of human origin. Later Kass and Seastone[57] reported experiments supplying further proof of the relation of hyaluronic acid to streptococcal virulence by demonstrating that mice could be protected against fatal streptococcus infection by treatment with hyaluronidase, an enzyme which hydrolyzes hyaluronic acid. The importance of hyaluronic acid in the virulence of Group C streptococci has long been accepted as an established fact.[65] A similar rôle for the same substance in the virulence of streptococci of Group A and other serologic groups would be in agreement with the observations already reviewed that non-Group A strains as well as all serologic types of Group A strains can produce disease. The capsular location of this substance in the cell, and the fact that autolysis of the capsule is associated with spontaneous phagocytosis in normal blood is additional evidence of its significance in the virulence of streptococci.[101] It is not impossible that hyaluronic acid exists in the cell in close association with the "M" substance; it being non-antigenic in itself, association with the "M" substance may render it a complete antigen.

The nature of the enzyme systems, both parasitic and host, involved in streptococcus infection also are receiving attention, and may yield valuable knowledge altering the basic concept of virulence. Elliott[28] recently described an enzyme occurring in certain streptococcus cultures which destroys the "M" substance produced by the culture when inoculated at body temperature. This observation, together with the studies on trypsin-antitrypsin systems[78] and the effect of antitrypsin on the growth of streptococci[79] suggest that autarceologic variations in the host may be of fundamental importance in understanding the nature of the phenomena usually designated as streptococcal virulence. It must be remembered, as Dubos[26] thinks, that bacterial virulence is not due to a single substance or phenomenon; but, rather, to a summation of the effects of many substances and phenomena in both the parasite and host.

Non-Group A Infections. The data reviewed here on the heterogeneity of serologic types as the etiologic agents of various streptococcus infections suggest that the disease-producing capacity of the hemolytic streptococcus is not dependent entirely upon differences in serologic type —or even group antigens, but more likely upon some common denominator which exists irrespective of serologic characteristics. Evidence is accumulating that the ability to reproduce severe, often fatal infection in man is not limited to Group A streptococci.[34,87,117] Non-Group A streptococcus infections, as compared to Group A

infections are admittedly rare, but do occur. Such infections usually are non-respiratory in origin. The ability of non-Group A strains to produce human infection is of interest, too, in that it raises further doubt on the long-disputed question of the rôle of the streptococcus toxins, erythrogenic factors, hemolysins, streptolysins and fibrinolysins in non-scarlatinal infection. With the exception of certain Group C[52] and Group G strains,[71] streptococci other than those belonging to Group A do not produce these substances, at least in measurable quantities (Table 3).

Table 3.—Production of Toxic Products by Various Serologic Groups of Streptococci

Serologic group	Production of erythrogenic toxin	Soluble hemolysin	Fibrinolysin	Streptolysin	
				O	S
A*	+	+	+	+	+
C	+	+	+	+	?
G	+	+	+	+	?
B, D, E, F, H, K†	0	0	0	0	?

* Includes various serologic types.
† Probably should include Groups L, M and N.

Table 4.—Production of Erythrogenic Toxin by Various Serologic Groups of Streptococci

Serologic group	Per cent toxic	Toxins neutralized by antistreptococcal horse serum (per cent of strains)			
		1	2	3	4
A*	83.7	75.7	11.0	6.2	7.1
C, G	9.4	0	12.5	0	87.5
B, D, E, F, H, K†	0	0	0	0	0

* Includes various serologic types.
† Probably should include Groups L, M and N.

In Table 4 it will be noted that although the majority of Group A streptococci produce an erythrogenic toxin, not all strains produce a toxin homologous with the N.Y.5 strain.[19] Although it is now well known that not all Group A streptococci produce an identical erythrogenic toxin,[55] there is no constant correlation between antigenic variety of toxin and serologic type. The heterologous toxin described by Trask and Blake[112] was produced by Type 18, while many Type 18 strains produce a toxin homologous with N.Y.5. As another example, in the experience of the authors, the majority of Type 6 strains produce a toxin neutralized by N.Y.5 antiserum, while occasionally a Type 6 strain is encountered which produces an heterologous toxin. As yet, there is no knowledge of the circumstances favoring toxin production or determining the antigenic variety of toxin produced. Aside from the ability of several serologic types (and groups) to produce an erythrogenic toxin, it appears from the work of Aranow and Wood[2] that certain strains of hemolytic *Staphylococcus aureus* are capable of pro-

The similarity between Group A and Group C and G strains is especially interesting in view of the fact that three of Griffith's original serologic types (Type 7, isolated from puerperal fever; Type 20, isolated from a carrier; and Type 21, isolated from scarlet fever) were found to belong to Group C, while a fourth (Type 16, isolated from puerperal fever) was found to belong to Group G.[47,65] The analogy goes further in that the toxin-producing Group C strains are of the "human" type, fermenting trehalose but not sorbitol, as is characteristic of Group A strains. Furthermore, Group G is not only biochemically similar to Groups A and C (trehalose positive, sorbital nega-

ducing a typical scarlatinal rash by the elaboration of an erythrogenic toxin which is antigenically related to that produced by the streptococcus.

MENINGOCOCCI. In spite of exhaustive bacteriologic and serologic distinctions, a question still confronts students of the epidemiology of meningitis which had already been raised and well expressed in the words of Hirsch written before the discovery of the meningococcus: "In a certain number of the epidemics that have been confined to the military . . . the earliest cases, and the majority of the cases throughout, occurred *among recruits*. It was natural to seek for an explanation of the fact in their altered mode of life, and more especially in the unwonted *bodily strain of their drill* or other duties of the service. Some . . . have gone so far as to make that factor the true and only cause of the disease. Other observers . . . have been less one-sided in the importance that they have assigned to these exactions of the service; and there can be hardly any doubt that they have had some effect in calling forth the disease. . . . It is clear that the significance to be assigned to that factor in the etiology is only that of a predisposing cause or an opportunity. It is much more reasonable, in my view, to connect the prevalence of meningitis epidemics among bodies of troops with their lodgment in more or less *crowded, badly kept, and insufficiently ventilated* tenements, or, in other words, with the same conditions that afford a peculiarly favorable soil for the development of this and many other infective diseases . . ."[54] (in more modern terms, increased opportunity for transmission resulting from the urbanization of nonimmunes as in measles).

The organism, *Neisseria intracellularis*, had been described by Marchiafava and Celli in 1884, but the significant study was that of Weichselbaum, who in 1887 described it in detail as the organism found in 6 cases of cerebrospinal fever. The substitution of actual laboratory tools and the development of the experimental method for the older method of observation now become speculation, gave great emphasis to the rôle of the infectious agent. As with many other infectious diseases, the discovery of a microbial etiologic agent "revolutionized our entire conception of its epidemiology" by displacing "the then dominant and paralyzing" theories of predisposition by the "more hopeful infective theory." As has not infrequently been the case in the pursuit of medical knowledge, the trend in research took the direction of the newer techniques—not necessarily that of the truth. The central idea in epidemiology became the infectious agent and its mode of spread, but the sporadic distribution of cases even in epidemics and especially the finding of meningococci in the nasopharynx of normal people as well as in cases, soon pushed the bacteriologic study of the disease beyond the mere question of exposure to the infectious agent. With the new techniques available, the explanation came to be sought in some variation or difference in the infective agent.

Differences in strains of meningococci were first recognized in the course of the preparation of therapeutic antimeningococcus serum. Several or many strains of the organism were employed for the inoculation of animals on the supposition that biologic variations in strains would be covered. It was the observation that strains of the organism isolated from the nasopharynx of healthy persons frequently were not agglutinated by sera prepared with strains from cases which led to attempts at classification into serologic types, first with a view to a better therapeutic serum and later from the point of view of a better understanding of epidemiologic relationships. That the earlier classifications represented only an imperfect separation of distinct types of the organism, and that they had little to do with pathogenicity is indicated by cross reactions between strains of different types, by a great lack of agreement be-

tween the classifications set up by different workers, and by the fact that all types could be found both in the clinical disease and in the healthy carrier.

It has been through a long process of careful selection of strains rather than the introduction of any new principle, that the present day classification of serologic types of meningococci has evolved. That this classification has meaning is shown by the fact that clinical meningitis is now associated in the vast majority of cases with a single serologic type, and that the incidence of the disease closely parallels the prevalence of this type of organism rather than that of the other three types which are distinguished. This does not mean, however, that the different serologic types are wholly unrelated in the epidemiologic sense, for when there is a change in type composition, an increase in the frequency of one type is accompanied by a corresponding decrease in the frequency of one or more of the other types. The relative increase in frequency of one type, for example, the increase in frequency of Type I meningococci which correlates with increase in the incidence of meningitis, does not appear to represent the "introduction of a new strain," but, rather, a differentiation of other types, more especially Type x into Type I; a change which occurs for reasons which are not known.

At about the time typing of meningococci reached the point of refinement which made it a differential procedure of any degree of precision, the advent of the newer chemotherapeutic agents went far to make typing no longer necessary, at least from the point of view of the purpose for which it was originally undertaken (the production of therapeutic serum). It has, nonetheless, turned out to be the only instance of serologic typing which constitutes a method of any epidemiologic importance in differentiating organisms that produce disease from those that do not. The present day understanding of the relationship between the prevalence of a single type meningococcus and meningitis not only throws into the discard some of the sweeping and laborious measures which had earlier been advocated as a *sine qua non* for the control of meningitis, particularly in the mobilization of armies; but revolutionizes some of the basic conceptions of the epidemiology of the disease.

The question whether meningitis is determined by "predisposing factors" or by "contagion"—raised even before the discovery of the meningococcus, gave way with the discovery of the meningococcus to studies centered entirely on the organism. Now the relationships between meningococcus carriage, in the light of serologic classification and the incidence of meningitis, reopens the same question in a somewhat modified form: *to what extent* is the incidence of meningitis determined by the dissemination of the organism, and *to what extent* is the disease determined by predisposing factors in the small portion of those who harbor a particular type of the organism? In other words, the strictly microbic point of view of a straight contest between host and infectious agent, begins to yield to the conception of a state of remarkable equilibrium between the host and parasite in which the disease occurs only exceptionally when this equilibrium is thrown out of balance by factors which, although not yet clearly understood, do not necessarily reside entirely in the parasite.

Soon after the agglutination test was introduced for the identification of meningococci, it was noticed that different strains possessed varying degrees of agglutinability. Kutscher in 1906 who employed the absorption test, observed that there was a marked difference between strains, but he was unable to classify them by this method.[60] In 1909 Elser and Huntoon found that 40% of meningococci, which they termed pseudomeningococci, were inagglutinable by a monovalent serum.[30] They further divided the pseudomeningococci by absorption into two sub-groups. In the same year, Dopter noticed the presence in nasopharyngeal mucus of cocci

resembling the meningococcus in morphology, cultural and fermentation reactions, but differing from it in their complete absence of agglutination with a meningococcal serum; these organisms he termed parameningococci.[23] Arkwright, also in 1909, noticed not only that by agglutination and absorption the organisms could be roughly divided into groups, but that, serologically, the sporadic strains tended to deviate more from the type to which most strains conformed than did the epidemic strains.[3] In 1914 Dopter and Pauron divided the parameningococci into 3 types,[24] and Ellis (1915) found that strains fell into 2 types, I and II, of which Type II was probably identical with Dopter's parameningococcus.[29] Arkwright (1915) was able to classify 30 out of 35 strains into 2 main groups, Types I and II,[4] of which Type II, like Ellis' corresponded to Dopter's parameningococcus; 3 strains were difficult to classify, and 2 were intermediate between the 2 types. Gordon and Murray (1915) found that strains from cerebrospinal fluid of epidemic cases fell sharply into 4 groups, which they called Groups I, II, III, and IV;[45] none showed any relation to Dopter's parameningococcus. In 1917, Nicolle, Debains and Jouan classified the meningococci into 4 types, called A, B, C, and D.[81]

Gordon's I and III strains corresponded to N., D. and J.'s Type A.
Gordon's II and IV strains corresponded to N., D. and J.'s Type B.
Dopter's meningococcus corresponded to N., D. and J.'s Type A.
Dopter's parameningococcus α corresponded to N., D. and J.'s Type B.
Dopter's parameningococcus β corresponded to N., D. and J.'s Type B or C.
Dopter's parameningococcus γ corresponded to N., D. and J.'s Type D.

F. Griffith (1917) found that meningococci could be divided into 2 main groups, I and II;[46] Group I corresponded roughly with Gordon and Murray's Groups I and III, and Group II with Gordon and Murray's Groups II and IV.

Wollstein observed that between the so-called normal and parameningococci there were a number of intermediate varieties.[120] According to Amoss, the studies of the meningococcus stimulated by the appearance of epidemic meningitis in the armies of all the belligerent powers in World War I brought meningococcus typing into a period of great activity. The general point of view of the time was represented by the classifications of Gordon and of Nicolle and their associates. Briefly stated they distinguished two main types of meningococci designated either Types I and II, or A and B. Gordon also recognized Subtypes III and IV, one affiliating with Type II and the other with Type I, and Nicolle likewise distinguished two such types, C and D, which showed similar affiliations.

As Amoss says, "The serological subdivision of the meningococcus into varieties or types has undoubtedly marked a forward step in our knowledge of the causation and specific treatment of epidemic meningitis. It may, however, perhaps be regarded as of questionable value to set up too many minor varieties or types."[1]

In Gordon's original investigation, 32 strains of meningococci were found to be resolved into 4 different groups. "As a rule, the results of simple agglutination were confirmed by the absorption test." There were "close affinities between members of Types 1 and 3, and 2 and 4, but absorption tests proved that these were due to minor or co-agglutinins, and that the major agglutinin of each of the four types was univalent and specific." Gram-negative cocci from the nasopharynx of nine contacts and one doubtful case of cerebrospinal fever were "submitted to investigation with the four univalent agglutinating sera that had been proved to include all of the serological types of meningococcus occurring in the cerebrospinal fluid of the thirty-two cases." All of these were "indistinguishable from the meningococcus in morphological, cultural, and fermentative characters." Six were found to be "serologically identical with

the meningococcus, five being specimens of Type 2, and one of Type 1." The remaining 4 could not be identified serologically with any of the 4 types of meningococcus. Two specimens were injected into rabbits and an agglutinating serum prepared against each of them. "The specific agglutinin of each of the pharyngococci, while readily removed by the homologous coccus, was unaffected by any of the four types of meningococcus obtained from the cerebrospinal fluid of the cases." Later specimens of meningococcus from the cerebrospinal fluid of cases were tested with the four univalent sera. "By the end of 1915 over sixty specimens of meningococcus had been examined with all four agglutinating sera, and found to be identical with one or the other of the four serological types."[43]

It hardly seems surprising that Gordon's classification based on the study of such a small number of cultures should have turned out upon further study to represent a far from complete separation of types of meningococci.

The combined force of the "new" type classifications, and the pressing needs of the military situation brought about the quick elaboration of a formula to explain the epidemic occurrence of the disease, and to meet the pressure for preventive measures. It was perhaps in part its plausibility and in part its preciseness which caused it to be so widely adopted. Observations made even in the course of these very studies which under ordinary circumstances would have raised doubt, passed seemingly unnoticed. It is furthermore curious that even though serologic typing so largely gave impetus to the epidemiologic studies concerning carrier rates, the epidemiologic deductions did not take into consideration at all the various serologic types. They were based rather on overall carrier rate.

The World War I concept had it that previous to an outbreak of meningitis, "carriers of the meningococcus increased steadily," from a "normal" carrier rate of from 2 to 4% to 20 or 30%, and that soon after it had passed the "danger point" of 20%, cases would begin to appear. A search of a very large literature of the time does not reveal any facts, or theory, for that matter, upon which a 20% "danger point" was based. There are statements concerning individual situations which, even if not of any particular significance from a statistical point of view, were so vivid that they were too readily incorporated in texts on meningitis. "The carrier-rate, which was 19.25 per cent . . . reached what is usually considered the danger point of twenty per cent (see War Office Memorandum on Cerebrospinal Fever, p. 2), just six days before the first case occurred."[39] Again, the carrier rate "which had been kept beautifully low during November (5 per cent. on November 29) now commenced to rise in ominous fashion; on December 6 it was nearly 17 per cent., on the 21st 19 per cent., just under the 20 per cent. danger line as laid down in the War Office memorandum." The idea was not in the least impeded by such lines as ". . . sporadic cases may admittedly arise with any carrier rate . . ."[40] and that 22% of civilians examined in an outpatient infirmary, "although they had no relation to cerebrospinal fever" harbored in their nasopharynx organisms indistinguishable from the meningococci,[100] a result confirming previous studies which showed that "meningococci are to be found in a considerable percentage of persons in whom no relation to cases of cerebrospinal meningitis is discoverable."

Having fixed a cause of epidemics with such a high degree of precision as the "warning rise" and "20% danger point," it was natural to hurry on to an explanation for these "sure storm signals of imminent danger." Investigating the cause of this "warning rise" in the carrier rate, Glover (1920) was led to suspect a relationship between it and overcrowding in the sleeping huts. These huts were "at the best poorly ventilated" (Eagleton,

1919–20), and during the stress of war "the mobilization standard had been overstepped," so that beds, instead of being separated, were practically touching each other. Glover (1920) noticed that the carriers in a given hut tended to be aggregated together. This pointed strongly to the direct transmission of the meningococcus from one man to another sleeping in the next bed. He tried the effect of spacing out the beds in the hope that the infection would be diminished. The results obtained "seemed to be in accordance with expectation." The effect of the distance between the beds was not confined to the carrier rate. At Caterham depot, "where there was severe overcrowding," an outbreak of cerebrospinal fever had occurred during each winter of the War, but subsequently to the adoption of the spacing-out policy in 1917–18, "not a single case occurred." It is sometimes difficult to determine whether in these studies, a high carrier rate was actually associated with overcrowding or was in itself interpreted as evidence of overcrowding. A sharp rise in the non-contact carrier rate "is as sure a storm signal of imminent danger of an outbreak as it is a sign of overcrowding or of dangerously deficient ventilation.[40] (The latter was sometimes invoked in instances of otherwise unsatisfactory evidence of "overcrowding.")

The far-reaching implications regarding the relation between overcrowding and the carrier rate; and that between the carrier rate and the disease; as well as the importance of detecting the "warning rise," were the cause of the wide acceptance of the whole doctrine, but there has been a noteworthy absence of confirmation of the main theses. In the Detroit epidemic of 1928–29, Norton and Gordon (1930) found no correlation between the degree of overcrowding in the home and the contact carrier rate.[82] During the outbreak of 1931 at Aldershot, Armstrong and his colleagues (1931), from a study of the position of carriers in dormitory barrack rooms, were unable to obtain any evidence that infection occurred mainly at night.[5] The carriers were scattered quite irregularly without any particular relation to the position of the beds. Other nasopharyngeal surveys of the civilian population, like that of Rake (1934),[86] have shown us that the carrier rate in institutions may be as high as 20% and over, without any outbreak of cerebrospinal fever occurring. Perhaps the most striking figures, however, are afforded by Dudley and Brennan (1934). Working at the Chatham naval hospital, they found that between January, 1932, and March, 1933, there were 11 cases of cerebrospinal meningitis with a carrier rate of about 13%. During the period, March, 1933, to May, 1934, the carrier rate was 54%, yet not a single case of meningitis occurred. During the same period at the Royal Naval Hospital, Portsmouth, there were 6 cases of meningitis with a carrier rate of only 5%. Analysis of the distribution of carriers at Chatham showed no constant relationship between the density of the population and the carrier rate. The senior ratings with the most spacious sleeping accommodation had as high a carrier rate—60%—as the recruits with the worst sleeping quarters.[27]

That all was not well with the World War I concept of meningitis and its prevention—that "when the carrier rate rises, it is direct evidence . . . of overcrowding; when that is remedied, the carrier rate automatically falls"[44]—is further supported by reports that "measures employed to prevent meningitis . . . cannot be considered as altogether successful. In spite of the great care exercised in the isolation of cases, wholesale examinations made to detect and eliminate carriers of meningococci, and the various other methods employed to limit the spread of the disease, the incidence of meningitis in troops was much greater than in the civilian population."[105] Thus, after the extensive researches of the World War I period, the status of serologic typing of meningococci, insofar as it provided any

epidemiologic explanation of the occurrence of the disease, or indicated methods of control, was much the same as that of the other groups of organisms considered in this paper. But as already stated, subsequent refinements of typing, notably the work of Branham, Rake, and Kirkbride have resulted in a method which does differentiate the "pathogen."

It was this advance, together with the advent of the newer chemotherapeutic agents with the possibility of their prophylactic use, which called for further studies of meningococcus carriage, when the World War II period again coincided with one of the periodic waves of meningitis prevalence (but not necessarily causally related).

The results of these studies, not yet available for complete review, will show it is believed, that the meningococcus carrier rate (all types) is the backlog upon which the carrier rate of the "pathogen" (Type I) smoulders. Within the relatively uniform overall carrier rate, the prevalence of Type I carriage rises and falls periodically, probably as a result of changes in type in the ordinarily less highly differentiated organism in the course of its dissemination, the cause of which is yet unknown. There are indications that periods of *relatively* high Type I carriage correspond to periods of increased prevalence of meningitis, and that the occurrence of the disease in the very small proportion of those who come to harbor Type I meningococci—larger in recruits than in seasoned troops or the civilian population—may be determined[7] "by the unwonted bodily strain of their drill or other duties of the service."[54]

Summary. Serologic techniques, perhaps the most valuable tools of the modern systematic bacteriologist, have made immeasurable contributions to the present-day knowledge of bacteria. As reviewed here, the application of type differences to the study of infectious disease also has increased certain phases of our knowledge concerning the bacteriology, epidemiology and therapeutics of infection. Type differentiation was of utmost importance, for example, before the advent of the sulfa drugs and antibiotics in the treatment of Clostridium infections, pneumococcus pneumonia, meningococcal meningitis and influenzal meningitis. Type differentiation, even in these days of penicillin and sulfa therapy and prophylaxis is still essential to the successful treatment of Clostridium intoxications, influenzal meningitis and in some cases of pneumococcal or meningococcal infection. The enteric fevers and the streptococcal infections are exceptions in that as yet serologic type seems to have no bearing on therapy, although in the case of enteric infection, type differentiation may be of some value in the selection of an appropriate sulfa compound for therapy.

From an epidemiologic viewpoint, serologic type differentiation is of some value in delineating the spread of a single strain under certain circumstances; for example, single source, institutional or family outbreaks of disease, and in the control of cross-infection on hospital wards. However, the idea that type differentiation would explain the epidemiology of infectious disease in general, or clarify the etiology of certain diseases in the sense of a single species for a single disease, has met with disappointment. With the exception of meningococcal meningitis, there is no instance of any degree of correlation between a single serologic type and a single disease process. Neither has the relationship of the carrier rate to clinical disease been established in any, other than meningococcal meningitis. The general similarity between the types of streptococci and pneumococci in normal carriers of these microörganisms and in the diseases they induce has contributed little to an understanding of the relationship between the case and the normal carrier, or in distinguishing between cause and effect with regard to case and carrier. The enteric group is perhaps slightly different in that by common agreement, all carriers

are considered as being potentially dangerous since all Salmonella, Eberthella and Shigella are potential pathogens. Even among the enteric group, however, serologic type affords no criterion for altering this concept by the selection and isolation or treatment of dangerous carriers.

Most disappointing of all, knowledge of serologic types has given us little insight into the basic mechanics of bacterial infection. Again, with the possible exception of the meningococcus, it is apparent that the ability of the respective microörganisms to produce scarlet fever, pneumonia, gas gangrene or enteric fever is entirely independent of type characteristics, unless one is willing to accept the thesis that in the Salmonella group, for example, there exists 164 separate type antigens each endowed with the same ability to incite disease in man.

Perhaps one of the fundamental faults in present-day bacteriology is our lack of a concept as to what constitutes a bacterial species. In an age of specialization, the bacteriologist is perhaps all too willing to assign the microörganisms to a specialized category, thereby overlooking for the moment the broader biologic phenomena common to a group of bacteria which actually may be of more fundamental importance than the criteria upon which a special category is selected. For example, where amongst the ponderous literature on the pneumococci, can be found any extensive studies on the observation that non-type-specific immunity can be produced against the pneumococcus, or on the host factors or host changes predisposing to lobar or bronchopneumonia?

As Brown[14] recently pointed out, "Every biologist assumes that species are relatively stable; they are the result of evolution which he has no reason to believe has been suspended, but which proceeds so slowly or so intermittently that it need not deter the giving of useful names to the species which he observes. Names are labels used for convenience in description and, if possible, to indicate genetic relationship." Should the concept of species be based on serologic or biochemical type; or should the broader view—the streptococci as a species, for example—be taken in an attempt to explain pathogenicity or "virulence," meanwhile retaining the biochemical and serologic types as convenient "labels?" In attempting to define "virulence" it should be remembered that the term is relative, in all probability, as Dubos[26] pointed out, depending upon a number of variables, not a single isolated substance or characteristic, but, rather, upon the entire spectrum of biologic behavior, both parasitic and host. The epidemiologic observations on the diversity of serologic types associated with infection which have been reviewed here, suggest that "typeness" *per se* is perhaps only one of the many variables which determine the ability of the microörganism to produce disease. It is indeed remarkable that the vast amount of knowledge resulting from study of serologic type has contributed so little to the knowledge of the basic processes involved in infection, or for that matter, recovery. Even in a disease as thoroughly studied as lobar pneumonia, the rôle of type-specific immune response in recovery from infection is still open to question.[88]

The frequent statement that evolution in science is paced by evolution in its methodology is so far from true in respect to serologic types of bacteria and the causation of disease that the question might well be raised whether the right methodology has yet been found. As Gallup had written in 1815 (long before the discovery of bacteria), "If instead of scrutinizing old shoes, rags, etc., to find imported fomites, and accusing foreigners of transporting epidemic disease, the zealous enquirers after truth had exerted their talents to discriminate and render innocuous the local causes of disease, the science of physic would have become less reproachful to its cultivators."[37] Perhaps not until equal attention has been directed to "typing" of autarceologic susceptibility

shall we understand those imbalances—which we call disease—in what may be, in a broader sense, a remarkable host-parasite equilibrium.

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PATHOLOGY AND BACTERIOLOGY

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THE SPREADING FACTORS AND BACTERIAL INFECTION

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IT is rather striking that in our interest in the processes involved in bacterial infection we have become so preoccupied with those factors, both cellular and humoral, which have been thought to lead to success or failure in combating such infections that the study of the site at which these factors exert their effect has been almost completely disregarded as being of minor significance. Surely the rôle of the connective tissue must be an important one in any final result because it is either a barrier that must be crossed or a pathway if pathogenic microörganisms are to advance from their point of entrance to more vital tissues.

In 1942 this neglect of a fundamental consideration in the study of the nature of bacterial infection was pointed out and its significance stressed in a most able review of the problems of connective tissue permeability by Duran-Reynals.[4h] It was appropriate that Duran-Reynals should draw attention to this concept, because his work stimulated the development of a new approach to the study of the mechanism of bacterial invasion of body tissues. Any such advance is to be welcomed because it is clear by now that the forces involved are more complex than originally appreciated and that a total understanding of what is involved can only come by a utilization of all available techniques as developed in both biological and physical sciences.

Before attempting to review our present knowledge as to the properties, nature and significance of spreading factors in bacterial infection, some consideration should be given to the position held by the connective tissue in this problem. In this connection the opinion that the ground substance of the connective tissue is a viscous substance offering resistance to the penetration of foreign materials, including bacteria and their products, would seem a reasonable one. It is also the opinion of Duran-Reynals and others that the permeability of the connective tissue is subject to variations which will in turn influence the ability of bacteria and other pathogenic matter to pass this barrier. While this permeability of the connective tissue is influenced by a number of diverse factors such as the age and race of the host and the area of the body concerned, as was pointed out by Duran-Reynals in 1936,[4f] it is now held by many that of all factors increasing this permeability those designated by the term "spreading factors" are the most important. On this basis there is reason for considering the action of such spreading factors as being of comparable importance to phagocytic cells and antibodies in the field of resistance, or lack of it, to bacterial infection.

The original discovery of the spreading factors and their present identification with enzymes capable of hydrolyzing hyaluronic acid has been the result of the

coming together of two apparently unrelated studies. During the course of experiments on the effect of inoculation of vaccinia virus in rabbits it was observed that infection would develop most rapidly and with greatest certainty when inoculation was made into the testis of the animal. This was reported in 1929 by Stewart and Duran-Reynals.[21] At this time Duran-Reynals also published reports[4a,b] in which he demonstrated that vaccinia virus infection of the rabbit was more effective when inoculated into the skin along with aqueous extracts of rabbit, guinea pig or rat testicle. It did not matter whether the testicular extract was injected along with the virus or was injected intradermally some time previously in the area selected for inoculation. This action of testicular extract in enhancing the virulence of the infecting agent was then shown to be the result of an effect exerted upon the host tissues and not upon the virus itself as could be proved by the fact that the virus recovered from such lesions showed no alteration in its infectivity. This fundamental discovery was soon followed by other studies using various infectious agents in several species of animals and in 1930 it was reported independently by McClean[8a] and by Hoffman and Duran-Reynals[7a] that this effect of testicular extract was due to an increased tissue permeability. The term spreading factors was applied to substances having this property and their occurrence in such varied sources as invasive bacteria, poisonous insects and snake venoms was described.[4e,g] In this connection it is interesting to note that observations have been reported[1,4c,16] concerning the presence of a spreading effect from tumor extracts when tested with vaccinia virus, India ink and diphtheria toxin. These reports are in agreement as to this effect, but they show disagreement as regards the spreading effect of a particular type of tumor. The original method employed in the study of spreading factors was to make intradermal injections of a material thought to contain the spreading factor plus an indicator, such as India ink, and to compare the spread of the inoculum with a control mixture. Since that time studies of the spreading factors in various tissues have been carried out and this effect is now reported for striated muscle, fasciæ, tendons, and gastric subserosal and subcutaneous connective tissues.

While this progress was being made on the mechanism of spreading factors, another series of investigations were being carried out in an apparently separate field. This work had to do with the nature of the ground substance in which the cells and connective fibers of the connective tissue are embedded. This matrix was generally assumed to be a protein of the "mucin" type, but protein-digesting enzymes such as trypsin are not spreading factors. In 1934 Meyer and Palmer[11] reported that the gelatinous fluid in vitreous humor is a kind of polysaccharide which they named hyaluronic acid. This discovery was followed in 1936 by a report by Meyer, Dubos and Smyth[12] that mucolytic enzymes could hydrolyze hyaluronic acid. Then in 1939 Chain and Duthie[2a] described the existence of a mucolytic enzyme in testicular extract which a year later they[2b] felt was identical with "spreading factor" and to which the term hyaluronidase was applied. In this manner two series of independent investigations were found to have a common bond of interest.

The acceptance of the identity of spreading factors and hyaluronidase is not complete. Duran-Reynals for example is inclined[4h] to place under the designation of "spreading factors" all those substances produced by living organisms which have the common property of increasing the permeability of the connective tissue. Under this inclusive definition spreading factors would tend to fall into two main groups. In one group would be placed those factors with spreading power *in vivo* and an enzymatic effect upon hyaluronic acid *in vitro*. In the second group would be substances, such as ascorbic acid, which have spreading power but which exert no

enzymatic activity on hyaluronic acid. At the present time the consensus is that the true spreading factors are those of the first group. These are the product of the simplest organisms to the more complex; they are very soluble in water; powdered preparations may remain active for years; they are destroyed by heating at 60° C. for 30 minutes;[7b] their effective permeability dose may be as small as 5×10^{-4} microgram;[8b] and in their action *in vitro* they are specific and only hyaluronic acid or its sulphuric acid ester from mesodermal structures is attacked. For these reasons it seems proper to regard the spreading factors as enzymes identical with the hyaluronidases.

The interest at present aroused in the antigenicity of enzymes, as exemplified in the studies of Seastone and Herriott[19] and of Sevag,[20] has focussed attention on this aspect of bacterial spreading factors. In 1932 Duran-Reynals reported[4d] that immune serum prepared in the rabbit against testicular extracts would precipitate the extracts, suppress their spreading power and neutralize their ability to enhance vaccinia virus infection. A more recent study by McClean[8e] showed that serum prepared against bull's testis inhibited this enzyme but did not inhibit a testicular enzyme from the mouse or any of the bacterial enzymes tested. This evidence of species specificity was in agreement with the results of other studies by the same author. In 1936 he had shown[8b] that whereas an immune serum could be prepared against *Clostridium welchii* which would inhibit the diffusing factor of that organism, it had no inhibitory activity against testicular extracts. Later studies by McClean and Hale[9] and by McClean[8e] have shown that antihyaluronidase serum would neutralize the activity of the enzyme in a specific manner and that immune sera prepared against enzymes obtained from *Cl. welchii* and *Cl. septicum* are species but not type specific, whereas those obtained against streptococcal enzymes are group but not type specific. Since the diffusing activity of these enzymes in the skin will occur at a much higher dilution than that at which any *in vitro* viscosity-reducing activity could be demonstrated and because the immune sera prepared against hyaluronidase was able to inhibit completely only a limited number of minimal diffusing doses of spreading factor in the skin, Sevag[20] is of the opinion that this is the probable reason for the failure of some experiments to demonstrate inhibition of the spreading factor. The specific character of the inhibition of the spreading effect has led Duran-Reynals[4h] to put forward two possible explanations for this phenomenon. On the one hand one can assume that the spreading factors are antigenic but quite different in even closely related species and this would indicate the presence of non-antigenic groups which would determine specificity. On the other hand one could speculate that the spreading factors are not antigenic and that the inhibition shown by immune sera is an accidental effect consequent upon a reaction with closely linked proteins which would inactivate the factors. The bulk of present evidence would appear to lend support to the first hypothesis.

Before considering the part that hyaluronidase may play in a specific bacterial infection, some discussion of the problem of invasiveness and/or, virulence is in order. With the majority of pathogenic bacteria in which invasive qualities are closely allied with virulence, we find that the characteristic picture of infection is one in which there is a well-recognized site of entrance into the body and a definite spread from that place. Staphylococcal, streptococcal and gas gangrene infections are of this order. In those infections in which the ability of the bacterial cells to cause disease is not so obviously allied with spread from a local portal of entry, we find that the invasion of the body tissues is one of dissemination by passive transfer from the respiratory or alimentary passages to those regions in which recognizable tissue changes occur, such as is the case in the majority of infections

caused by *Mycobacterium tuberculosis*, *Neisseria intracellularis* (meningococcus) and *Eberthella typhosa*. Studies[4e,8b] that have been made concerning the production of spreading factor by individual bacterial species tend on the whole to be correlated with the significance that this property would appear likely to exert on the life history of a particular type of bacterial infection; but this correlation of spreading factor and invasive power is only a rough one at best and does not correspond to any other method of classification of pathogenic bacteria.

The part that spreading factor production plays in staphylococcal infections is very definite. Duran-Reynals[4e] is of the opinion that *Staphylococcus pyogenes* can be further subdivided into groups on the basis of spreading factor production which will correspond to the quality and size of the lesion produced when these organisms are inoculated intradermally into rabbits.

The picture presented in streptococcal infections is not nearly so definite. In fact, while some strains of streptococci possess the spreading factor, others lack this ability and indeed the substrate to it (hyaluronic acid) is present in the capsule of some species. In 1944 Crowley[3] reported a study of hyaluronidase production by hemolytic streptococci from human sources. She found that in 308 strains of Lancefield Group A organisms 64 strains, but from only two serological types (4 and 22), showed hyaluronidase activity; but in 68 strains of Groups C and G streptococci 48 strains showed this property. In this particular study all hyaluronidase-producing strains were non-capsulated. Earlier reports[8d,13] had shown that it was possible to obtain an enzyme from the culture filtrates of some hemolytic streptococci which would lyze streptococcal capsules. While McClean[8d] suggests that this enzyme is identical with the hyaluronidase produced by these organisms, Hobby *et al.*[6] found that though some preparations from Group A hemolytic streptococci possessed both spreading factor and the ability to hydrolyze hyaluronic acid, preparations from other Group A streptococci possessed the spreading factor but failed to hydrolyze hyaluronic acid. This leads to the conclusion that invasiveness of hemolytic streptococci involves factors other than hyaluronidase and may explain the divergence of opinion between Duran-Reynals,[4e] who thought that the degree of invasiveness was largely determined by the amount of spreading factor, and Crowley[3] who found no evidence that hyaluronidase-producing strains were associated with any particular type of infection or that hyaluronidase production is related to the virulence of streptococci for man.

The studies of Goodner[5a,b] dealing with the production of skin lesions in the rabbit by the pneumococcus have shown that the injection of pneumococcal autolysates results in large spreading lesions and that such autolysates, from both homologous and heterologous types, would enhance the lesions produced in the rabbit by Type III pneumococci of varying pathogenicity.

In 1936 McClean[8b] reported that the culture filtrates of certain pathogenic clostridia contained a spreading factor and more recently he[10] has produced additional evidence to give substance to the claim that those clostridia which are characterized by their capacity for invasion of tissues are able to elaborate hyaluronidase. As a result of the study of the production of hyaluronidase by the clostridia, McClean, Rogers and Williams[10] have made a novel suggestion concerning its possible use as a diagnostic procedure. It was appreciated that it might prove of considerable value to be able to demonstrate the presence of actively multiplying microorganisms before their presence can be detected by usual methods of clinical or laboratory examination. This is particularly true of gas gangrene infections where the need for early surgical interference is imperative. It was their belief that the demonstration of bacterial enzymes in the wound exudate would provide a means whereby this early diagnosis could be made. The work reported is purely ex-

perimental in nature and no human gas gangrene infections were available for study. However, they found that in guinea pigs it was possible to establish infection with the use of pure cultures of *Cl. welchii*, *Cl. septicum* and *Cl. novyi*. In the case of strains producing hyaluronidase, it was possible to demonstrate this enzyme in the edema fluid as soon as sufficient could be collected for examination and in the muscle as soon as the earliest signs of infection appear. It is their contention that the presence of hyaluronidase can be detected by the Mucin-Clot Prevention test and that this test can be carried out in any laboratory with ordinary equipment. On the basis of their work they concluded that a large proportion of *Cl. welchii* strains produce hyaluronidase as well as lecithinase, which is identical with the alpha toxin. All strains of *Cl. septicum* examined produced hyaluronidase, but in the case of *Cl. novyi* less than half of them produced the enzyme and there was no evidence that this property was correlated with the ability of this organism to produce gas gangrene. These experimental results were obtained using pure cultures of the respective infecting organism. In natural gas gangrene infection, one would justifiably expect a mixed bacterial flora to be present which would contain hyaluronidase-producing organisms other than clostridia. In spite of this obvious lack of specificity in hyaluronidase production as a diagnostic criterion, it would seem possible that it may prove a useful adjunct in the making of an early diagnosis.

In infections produced by *Corynebacterium diphtheriæ* it has long been held that invasion of tissues by the organism does not occur and that virulence is entirely dependent upon the ability of the infecting strain to secrete toxin. The appearance in Europe in the last 20 years of a much more severe form of diphtheria has led to a necessary review of the whole problem in the hope that a fresh approach would lead to greater understanding of the underlying mechanism. While the majority of investigators are agreed that the toxins secreted by all strains of *C. diphtheriæ* are identical,[17,22] though the amount produced may vary with the growth environment as suggested by Mueller,[14a,b] O'Meara[15] is of the opinion that diphtheria toxin is composed of 2 fractions whose relative amounts vary with the individual organism. O'Meara is of the impression that his A fraction of diphtheria toxin is analogous to spreading factor. However, McClean[8c] studied 50 strains of diphtheria bacilli and found that only a few of them had a spreading effect of moderate intensity and that only one had a detectable enzymatic activity. It should be noted that some investigators[18] are of the opinion that gravis strains of *C. diphtheriæ* are more invasive than others, and there is evidence[1,4c] that the presence of spreading factor will accelerate the passage of diphtheria toxin from its site of production. In this connection it would seem reasonable to speculate that the more severe clinical symptoms presented by combined infections may at least in part be due to the elaboration of spreading factor by the accompanying organism.

In summing up our present knowledge concerning the place of the spreading factor in bacterial infection, it is possible to draw some definite conclusions in spite of the very considerable amount of investigation that remains to be done. In the first place this is a new approach to an old problem that has shown itself worth the attention devoted to it. On this basis it would appear certain that increased attention will be directed again to this aspect of bacterial infection when the present all-absorbing interest in chemotherapy and antibiotics adjusts itself with the passage of time to more equable levels. Though there are likely a number of spreading factors, that which has been identified with the enzyme hyaluronidase would seem to be of major significance. In this respect the concept expressed by Duran-Reynals[4h] is of interest. He is of the opinion that, whereas the other bacterial weapons employed in the invasion

of tissues are directed against barriers, such as the mobilization of phagocytic cells and the laying down of a fibrin clot—phenomena which are called forth by the appearance of the invading organism—the spreading factor acts upon a preëxisting physiologic barrier. The corollary to this is that hyaluronidase production is probably the first force called into action by those organisms able to secrete it in their attempt to pass through the connective tissue. From that point of view which is primarily concerned with applying our understanding of the mechanism of bacterial infection to the prevention or control of such processes, it would seem that the study of the immunologic aspects of hyaluronidase should yield information of value. While it seems unquestionable that hyaluronidase has antigenic properties, the results obtained with immune sera prepared against it do not as yet warrant any forecast as to its possible employment in therapy. At the present time one can only hold to the opinion that any increase in fundamental knowledge will almost inevitably lead to the development of procedures of practical value.

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PHYSIOLOGY

PROCEEDINGS OF
THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA
SESSION OF JANUARY 15, 1946

Absence of Antibodies in Granulocytes and Macrophages During Antibody Formation. W. E. EHRICH, M.D., T. N. HARRIS, M.D., and E. MERTENS, M.D. (Philadelphia General Hospital, Children's Hospital, Philadelphia, and Depts. of Pathology and Pediatrics, Univ. of Penna.). A comparative study was made (1) of the relative agglutinin titers in the foot pads, the popliteal lymph nodes, and the blood serum of rabbits that had received injections into the foot of dysentery vaccine with or without paraffin oil; and (2) of the titers in the granulocytes and macrophages, and the supernatant fluid of the peritoneal exudates of rabbits injected intraperitoneally with various dysentery and typhoid antigen combinations. Although there was excellent antibody response, the exudates in the foot pads and the peritoneal cavity showed only insignificant quantities of agglutinin; in fact, the isolated granulocytes and macrophages of the peritoneal exudates revealed no agglutinin at all. As similar results were obtained by intravenous injection of antibody and subsequent introduction of a non-specific irritant, it was concluded that the low antibody titers at the sites of injection were due to secondary concentration here of antibody that was synthesized in the regional lymphatic tissue; there remained nothing to suggest that either the granulocytes or the macrophages were instrumental in the synthesis of the agglutinins studied.

The Fate of Particulate Antigens in Relation to the Formation of Antibody. T. N. HARRIS, M. D., E. MERTENS, M.D., and W. E. EHRICH, M. D. (Children's Hospital of Philadelphia, the Philadelphia General Hospital and the Depts. of Pediatrics and Pathology, School of Medicine, Univ. of Penna.). The fate of particulate antigenic material has been investigated, in the period between its injection into the foot of the rabbit and the appearance of antibodies in the regional lymphatic tissue.

It has been found that a soluble substance immunologically identical with the antigenic material injected can be identified in extracts of the injected tissue and of the regional lymph node, and in the efferent lymph from that node. The concentration of this soluble material falls off slowly in the injected tissue in the course of the few days following the injection. In the extract of the lymph node and in the lymph it falls off quickly, and is succeeded by the appearance of antibody.

Evidence is presented that this material is derived from the antigenic material injected by a physiologic process, and some quantitative implications are discussed.

Blood Flow in the Bronchial Artery of the Anesthetized Dog. H. D. BRUNER, M.D., and C. F. SCHMIDT, M.D. (Dept. of Pharmacology, Univ. of Penna.). Blood flow in the right bronchial artery was measured by the bubble flowmeter in 36 dogs anesthetized by pentobarbital or chlorolose, in order to evaluate possible causal relations between this systemic blood flow and production of paroxysmal pulmonary edema. In 12 control experiments, flow was highest at the beginning of an experiment and decreased with decline of systemic pressure and cardiac output probably due to loss of heparinized blood. The average of the highest observed flows was 8.81 cc. per min., range, 1.1 to 26.7 cc. per min. The average maximal flow in 10 dogs ventilated by pump was

9.9 cc. per min. compared with 10.8 cc. per min. when breathing spontaneously. Several showed temporary cyclic or sporadic variations of flow without change of experimental conditions. In terms of simultaneous cardiac output, the extremes of flow were 0.03% and 0.8% of the cardiac output, and perhaps 50% goes to extrapulmonary structures; since the right lung is 1.25 times as heavy as the left, observed flow times 0.9 would represent approximately the order of flow which would drain into the pulmonary veins or enter the pulmonary artery capillaries on the respiratory bronchioles. Stimulation of the peripheral cervical vagus was followed by increased flow despite fall of systemic blood pressure, whereas stimulation of the peripheral cardiac branches from the stellate ganglion resulted in decreased flow despite raised systemic blood pressure. Acetylcholine, mecholyl, histamine, the xanthines, and nitroglycerine were dilator substances on intra-arterial injection, while epinephrine, ephedrine and posterior pituitary extract were constrictor. Atropine blocked the dilator action of acetylcholine, but only partially blocked that from cervical vagus stimulation. Ventilation with 7% CO_2 resulted in a complex response, but the local effect of blood with this CO_2 content appeared to be dilator; a low PO_2 (7.5% O_2) appeared to be weakly dilator in local action. The data are not consistent with the supposition that excessive bronchial artery flow is a direct cause of paroxysmal pulmonary edema.

Electrokymography Utilizing the Fluoroscope. W. EDWARD CHAMBERLAIN, M.D., BERT R. BOONE, M.D., and GEORGE C. HENNY, M.D. (Depts. of Radiology and Medical Physics, Temple Univ. Medical School and U. S. Public Health Service). The 931–A photo-multiplier tube, used with a recording galvanometer, will accurately record variations in light intensity at the photo-sensitive surface of the tube. Such variations in light intensity may be produced in various ways from physiologic motions in the human or animal body. With proper application these motions are recorded with fidelity (Am. J. Roentgenol., **54**, 217–229, 1945). For example, the motion of a selected point on the border of the fluoroscopic silhouette of a patient's heart may be recorded. This is done by placing a small piece of fluorescent screen directly over the light aperture of the photo-tube. The fluoroscopic Roentgen ray beam, after passing the selected part of the heart border, traverses a series of apertures in a simple arrangement of lead diaphragms and falls upon the fluorescent screen. The motion of the heart border produces variations in the amount of light emitted by the screen. Resultant variations in the electrical output of the photo-tube are readily recorded by a string galvanometer on moving bromide paper.

Other applications, with and without the fluoroscope, readily suggest themselves. For example, very successful plethysmographic records have been obtained.

Roentgen kymography has been used for the study of heart border motion but has failed to satisfy all of the requirements. Electrokymograms resemble electrocardiograms in detail and amplitude. Because they are accomplished with fluoroscopic Roentgen ray beams of conventional intensity, records of any desired length may be obtained. By simultaneous recording of the carotid pulse on the same strip of bromide paper as the electrokymogram, interpretation of the record of heart border motion is greatly facilitated. Experience suggests that electrokymography may find a field of usefulness in clinical cardiology as well as in physiologic and pharmacologic research.

BOOK REVIEWS AND NOTICES

PHYSICAL CHEMISTRY OF CELLS AND TISSUES. By RUDOLF HOBER, University of Pennsylvania School of Medicine, Philadelphia, Pa. Pp. 676; 70 ills. Phila.: Blakiston, 1945. Price, $9.00.

IN this volume physiology is considered "as a branch of physical chemical science dealing with life as a physical, though exceedingly complex system, that may be subjected to scientific analysis like any other natural object." The book begins with a discussion of the fundamentals of classical physical chemistry, commencing with atomic and molecular structures and graduating to more complicated aggregations of these basic units, such as the amorphous colloidal micellæ. Next, the structure of protoplasm is considered and the functional aspects of plasma membranes. Cell and tissue respiration, contractility, intestinal absorption, and the formation of urine are among the subjects treated. Of great interest is the discussion of the mechanism by which, in submicroscopic cellular structures, the liberated energy can be utilized for the performance of work.

Dr. Hober is one of the best known workers and teachers at the Marine Biological Laboratory at Woods Hole and is a member of the Department of Physiology at the University of Pennsylvania. Dr. Hober was dismissed as President of the University of Kiel for his activity in opposing Nazism and his books were among the first to be burned.

This present volume is an excellent modern treatment of the subject, filled with stimulating ideas, and soundly objective concepts. It should be read by everyone engaged in work on the cell. D. C.

THE PSYCHOANALYTIC THEORY OF NEUROSIS. By OTTO FENICHEL, M.D. Pp. 703. New York: W. W. Norton, 1945. Price, $7.50.

INSTEAD of complying with the request for a second edition of "Outlines of Clinical Psychoanalysis," the author has prepared the present book, which is only intended for those who have undergone a personal analysis. No definite case reports are given but there are examples to illustrate different mechanisms. Part I: Preliminary Considerations: Introductory Remarks on Psychoanalysis and the Theory of Neurosis; The Dynamic, The Economic, and The Structural Point of View; The Method of Psychoanalysis; Early Mental Development: Archaic Ego; Early Mental Development (Continued): Development of Instincts, Infantile Sexuality; Later Phase of Development: The Superego. Part II: Psychoanalytic Theory of Neurosis: Traumatic Neuroses; The Motives of Defense; The Mechanisms of Defense; The Direct Clinical Symptoms of the Neurotic Conflict; Anxiety as Neurotic Symptom: Anxiety Hysteria; Conversion; Perversions and Impulse Neuroses; Depression and Mania; Schizophrenia; Defenses Against Symptoms, and Secondary Gains; Character Disorders; Combinations of Neuroses and Psychoneuroses; The Clinical Course of Neuroses; Therapy and Prophylaxis of Neuroses.

Indications for Psychoanalytic Treatment: hysteria, compulsion neuroses, and pregenital neuroses, "neurotic" depression, perversions, addictions, and impulse neuroses, psychoses, severe manic-depressive cases, and schizophrenics. Contraindications: the age, feeblemindedness, unfavorable life situations, the triviality of neuroses, the urgency of a neurotic symptom, severe disturbances of speech, lack of a reasonable and coöperative ego, certain secondary gains, schizoid personalities, contraindications to analysis with a particular analyst. In the manic-depressive and schizophrenic groups, it is better for patients and for the benefit of science for psychoanalytic studies to be conducted in institutions. The very brief discussion on shock therapy states that such treatments should be administered after psychotherapy has proved unsuccessful. The book represents the most approved and advanced psychoanalytic thought. There is a bibliography of 74 pages. N. Y.

ELECTROTHERAPY AND LIGHT THERAPY. With the Essentials of Hydrotherapy and Mechanotherapy. By RICHARD KOVÁCS, M.D., Professor of Physical Therapy, New York Polyclinic Medical School and Hospital, etc., New York. Pp. 694; 353 ills. Fifth ed. Phila.: Lea & Febiger, 1945. Price, $8.50.

THIS edition is a triumph both for Kovács and the publishers. The first thing a reviewer notices about this new volume is the snugness with which it fits into the hand as compared with the bulkier volumes of former editions. In these days of acute shortages, it would be difficult to ascertain by what legerdemain of the book binding and printing arts this book has been produced. The excellent paper and the clarity of the print and illustrations make this book a joy to read. Incidentally, there are 38 more photographs and schematic drawings than in the previous edition.

The section on Static Electricity appears to be the only one that has been abbreviated. This seems unfortunate as the static machine is a valuable aid in the hands of one trained to use it. More physicians should emulate the late William Benham Snow, who obtained remarkable results with static electricity. It is true that the stigma of empiricism attaches to many of the treatments of physical therapy in the minds of many physicians, and this is well exemplified by the use or abuse of the static machine. However, many bio-physists are endeavoring to set up standard methods of measurements and physical and physiologic bases for treatment. Kovács' expanded section on Newer Methods of Electro-Diagnosis and Electromyographic Diagnosis gives an indication of what is being attempted along the lines of measuring action potentials accurately. Investigations such as these will rid physical medicine of many of its rule-of-thumb methods.

Behrend's chapter on Exercise is also considerably enlarged, and there is an excellent review of the modern physical therapy treatment of infantile paralysis with an unbiased evaluation of Sister Kenney's methods. These two additions are welcome, as more careful attention should be paid to corrective exercises and muscle re-education.

A glossary in two parts has been added to this fifth edition. Part I is of terms used in Electrophysics and will prove valuable to the physician and technician using electrical apparatus and for an understanding of the subjects of Electrophysics. Part II is of terms relating to Mechanotherapy and Muscle and Nerve Action.

It is not too much to say that no other book will so logically and easily induct the physician into the mysteries of medical electrophysics as this fifth edition of Kovács' *Electrotherapy and Light Therapy*. This volume ought to be in the library of every physician and is absolutely essential to physicians specializing in Physical Medicine and their technical aides. S. H.

AN INTRODUCTION TO PHYSICAL ANTHROPOLOGY. By M. F. ASHLEY MONTAGU, Associate Professor of Anatomy, Hahnemann Medical College and Hospital, Philadelphia; Visiting Lecturer, Department of Sociology, Harvard University. Pp. xiv, 326; 25 figs.; 6 tables. Springfield, Illinois: Charles C Thomas, 1945. Price, $4.00.

THIS excellent little volume provides a most readable and stimulating approach to the subject-matter of physical anthropology. It is not intended to serve as a text nor a reference work for the specialist but rather as an introduction to a fascinating and important field. The general student, be he physician, anatomist, sociologist, or psychologist, will discover numerous facts and ideas in its chapters which should afford him an even deeper appreciation of his own particular subject. The text is systematic, well organized and clearly written and the tables and diagrams are ingeniously arranged and informative. The author is himself a well known anthropologist and anatomist and should speak with authority on the subject.

In the first 2 chapters definitions and general principles are discussed and a brief but systematic account is given of the primates as a zoölogical group. Chapter III deals with the origin and evolution of the non-human primates and provides a necessary background for an understanding of the more recent evolution of man himself. Evidence is given that the tarsioid group provided the material from which all later primates developed. Chapter IV is concerned with the advent of man himself and the fossil types, particularly the more recent discoveries, which point the way backwards along

the zigzag path into the past. Newer theories concerning present day man's evolution reflect considerable doubt upon the older concepts of linear evolution and attention is drawn to the almost certain admixtures which must have occurred between extinct *Homo sapiens* and other coeval human types in the past. Chapters V and VI give a progressive and scientific basis for an analysis of the various present day ethnic groups. Shibboleths and misconceptions of "race" are strongly debunked. True genetic criteria are rightly advocated as the only scientific means by which group affinities and descents may be ascertained. The last 2 chapters might be said to deal with the scientific ideology which should furnish the basis for any true and proper evaluation of the physical and mental "protuberances" of man, taken individually or in groups. An appendix gives some of the more common methods and procedures used in anthropometry.

A rudimentary acquaintance with the principles of anthropology will do no one any harm, least of all the doctor. Alarming misconceptions held by many medical students concerning both fact and fancy of race, have convinced the reviewer (himself no anthropologist) that this book should be required reading for every would-be physician. J. P.

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